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普罗托品对家兔血小板功能的影响

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Effect of protopine on rabbit platelet function

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ABSTRACT Protopine (Pro) inhibited dose-dependently rabbit platelet aggregation induced by ADP, arachidonic acid (AA), collagen, or aggregoserpentin of *Trimeresurus mucrosquamatus* venom (TMVA) *in vitro*. Their IC_{50} were 25.3, 30.5, 46.9, 33.4 μmol·L^{-1}, respectively. Pro 10, 20 mg·kg^{-1} iv also inhibited the platelet aggregation induced by these inducers. The effects (maximal at 5 min) lasted 1 h.

By using fluorophotometry and RIA, it was seen that Pro suppressed the release of 5-HT from platelets during aggregation induced by collagen, AA, or TMVM *in vitro*. Pro did not block the formation of thromboxane A_2 during aggregation induced by AA and did not increase the content of cAMP in rabbit platelet, but increased the content of cGMP in rabbit platelets.

The antiplatelet effect of Pro may be related to an increase cGMP in rabbit platelets and the suppresson of the release of the active substences from platelets.

KEY WORDS protopine; platelet aggregation; thromboxane A_2; cyclic AMP; cyclic GMP; adenosine diphosphate; arachidonic acid; collagen; snake venoms

摘要 普罗托品(protopine、Pro)体外(1—1000 μmol·L^{-1})和体内(10和20 mg·kg^{-1})均抑制ADP，胶原，花生四烯酸(AA)和烙铁头蛇毒血小板聚集素(TMVA)诱导的兔血小板聚集及血小板5-HT释放. Pro不抑制AA诱导的兔血小板TXA_2生成. 也不升高血小板内cAMP水平，但升高cGMP水平. 提示其抗血小板作用的机制与升高血小板内cGMP水平，抑制血小板释放活性物质有关.

关键词 普罗托品；血小板聚集；血栓素A_2；环腺苷-磷酸；环鸟苷-磷酸；腺苷二磷酸；花生四烯酸；胶原；蛇毒

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普罗托品(Pro)常与异喹啉类生物碱共存于罂粟科(Papaveraceae)、蓝堇科(Fumariaceae)植物中[1]. 具有与异喹啉类生物碱相似的药理作用如松弛胃肠道平滑肌[2]、抗炎抗肿瘤及抗心律失常[3]等. 本室在进行抗血小板聚集药物筛选时发现Pro具有较强的抑制血小板聚集作用，这是异喹啉类生物碱所少有的. 这方面的作用尚未见报道. 血小板的活化聚集与血栓形成在动脉粥样硬化中起着重要的作用[4]. 寻找抗血小板聚集作用的药物对于心脑血管疾病的防治有重要的意义. 本文研究Pro的抗血小板聚集作用.

MATERIAS AND METHODS

醋酸普罗托品(protopine acetate, Pro)，由大理医药公司刘晓初工程师提取惠赠，纯度99 %；咪唑(imidazole, Imi，瑞士Fluka)临用时用0.2 mol·L^{-1}磷酸缓冲液配制pH 7.1；茶碱(theophylline, TP，上海试剂二厂，批号850125)；硝普钠(sodium nitroprusside, SN，武汉第二制药厂，批号7705 10)、二磷酸腺苷(adenosine diphosphate, ADP)、胶原(collagen)均美国Sigma生产；烙铁头蛇毒血小板聚集素(aggregoserpentin of *Trimeresurus mucrosqamatus* venom,

TMVA)，中国科学院昆明动物研究所提供；花生四烯酸 (arachidonic acid，AA)，5-羟色胺硫酸肌苷 (5-hydroxytryptamine creatinesulfate，5-HT)和邻苯二甲醛(*o*-phthalialdehyd，*o*-Pht)均为瑞士 Fluka；TXB_2放免药盒，中国医学科学院基础所提供；cAMP 和 cGMP 放免药盒，中国科学院原子能研究所提供. 兔体重2.2±*s* 0.3 kg，♀♂兼用，本院动物科供给. 血小板聚集仪(BS-631型，北京生化仪器厂)；荧光分光光度计(MPF-4，Hitachi)；液体闪烁计数器(1217型，LKB).

体外血小板聚集实验 兔6只，颈动脉放血，3.8 %枸橼酸钠抗凝，血与抗凝剂之比为9:1 (vol/vol)，离心分离富血小板血浆(platelet-rich plasma，PRP，150×*g*，10 min)及贫血小板血浆(platelet-poor plasma，PPP，1200×*g*，10 min). 以 PPP 调整 PRP 中的血小板数至500×10^9 platelets·L^{-1}. 按比浊法[5]进行血小板聚集性测定，取0.45 ml PRP 与不同浓度 Pro 0.05 ml 或 NS 于37 ℃温育5 min 后分别加入 ADP (10 μmol·L^{-1})，胶原(10 μg·ml^{-1})，AA (100 μg·ml^{-1})和 TMVA (0.22 μmol·L^{-1})诱导血小板聚集，药物对血小板聚集的抑制率按下式计算：

聚集抑制率(%)=[(对照聚集%－药物聚集%)/对照聚集%]×100 %

体内血小板聚集实验 兔分3组，每组6只，分别经耳 iv Pro 10和20 mg·kg^{-1}以及等容量 NS，于给药前及给药后5，20，40，60和120 min 自颈动脉取血制备 PRP 及 PPP，测定血小板聚集性，测定方法同体外试验.

血小板5-HT 释放反应[6] 做完聚集实验后用盐酸1 mol·L^{-1} 0.1 ml 终止反应，置冰浴离心(1200×*g*，10 min)，取上清液0.4 ml 加 Triton X-100 0.1 ml 及酸化正丁醇3 ml 提取5-HT，在正丁醇提取液中加入正庚烷5 ml 和0.1 %半胱氨酸1 ml，离心，取水相0.5 ml 加0.004 % *o*-Pht 3 ml，置沸水浴15 min，冷却后进行荧光测定(λ_{ex} 365 nm，λ_{em} 480 nm). 测定管(PRP)结果减去对照管(PPP)，并与同样操作的5-HT 标准管比较，求得血小板5-HT 含量.

血小板 TXB_2测定[7] TXB_2是 TXA_2的代谢产物，但 TXA_2半衰期短($T_{1/2}$=30 s)，因此本文测定 TXB_2的量以间接反映 TXA_2的生成情况. 取 PRP 0.05 ml 加药液或对照液0.45 ml，37 ℃温育5 min 后，以 AA 为诱导剂诱导血小板聚集，用盐酸 1 mol·L^{-1}终止反应，离心(1200×*g*，10 min)，取上清液0.4 ml 加石油醚5 ml 离心，弃上层液，加重蒸馏 EtAc 5 ml 提取两次，合并提取液，负压抽干，－15 ℃保存. 测定前用磷酸缓冲液将样品复溶，用放免法测定 TXB_2含量.

血小板内 cAMP 及 cGMP 含量的测定[8] 取0.9 ml 血小板悬液，分别加入 Pro，TPSN 或 NS 0.1 ml，37 ℃水浴15 min. 加等容量15 %三氯醋酸，静置2 h，使血小板充分破坏，离心(1200×*g*，10 min)，沉淀部分按比色法测定蛋白质含量. 上清液用五倍体积的水饱和乙醚提取3次，除去三氯醋酸及乙醚层，最后取0.5 ml 提取液37 ℃蒸干，用放免法测定 cAMP 及 cGMP 含量.

RESULTS

对兔血小板聚集的影响 Pro 对 ADP、胶原、AA 和 TMVA 诱导的离体兔血小板聚集均有抑制作用，并与剂量相关，IC_{50}及95 %可信限分别为：25.3 (20.3－31.6)，30.5 (24.6－37.8)，46.9 (38.4－57.2)，33.4 (27.0－41.3) μmol. L^{-1}(Fig 1).

Pro iv (10，20 mg·kg^{-1})对上述四种诱导剂诱导的兔血小板聚集也有抑制作用，给药后5 min 显现效应，持续约60 min，2 h 后作用消失；给兔 iv 等容量的生理盐水对上述四种诱导剂诱导的血小板聚集无影响(Tab 1).

对血小板5-HT 释放反应的影响 Pro 在抑制 AA、胶原和 TMVA 诱导的兔血小板聚集时也抑制5-HT 释放，抑制作用与剂量相关，以 ADP 为诱导剂未见兔血小板5-HT 释放(Tab 2).

对 AA 代谢的影响 Pro 在抑制 AA 诱导兔血小板聚集时并不抑制 TXB_2生成，而咪唑 (30 mmol·L^{-1}) 可完全抑制 TXB_2生成(Tab 3).

对血小板内 cAMP 及 cGMP 水平的影响 在体外抑制血小板聚集的浓度下，Pro 并不升高血小板内 cAMP 水平，而是升高 cGMP 水平，其作用较 SN 弱；阳性对照药 TP 可显著升高 cAMP 水平(Tab 4).

Tab 1. Effects of protopine on platelet aggregation induced by ADP, collagen, AA, or TMVA *in vivo*. $n=6$ rabbits, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 min.

Inducer	Protopine/ mg·kg^{-1}	Platelet aggregation/% 0	5	20	40	60	120 min
ADP	0	53.2±2.5	52.4±1.8^a	53.1±2.4^a	51.9±3.0^a	52.9±1.5	52.5±1.3^a
	10	53.6±1.6	24.7±2.9^c	27.8±1.8^c	28.6±4.0^c	40.7±3.6^b	52.0±2.0^a
	20	52.9±2.5	11.4±1.7^c	11.7±2.3^c	16.9±1.6^c	29.9±3.1^b	50.9±2.3^a
Collagen	0	58.0±2.5	57.5±2.7^a	56.9±2.4^a	57.0±3.2^a	57.5±1.6^a	58.0±1.3^a
	10	57.9±3.2	26.3±3.9^c	27.7±2.1^c	34.7±1.3^c	35.9±3.5	55.9±2.5
	20	58.0±1.4	14.3±3.3^c	16.0±4.2^c	22.2±7.6^c	35.8±3.2	55.5±4.5^a
AA	0	66.3±1.3	65.5±2.5^a	65.3±1.3^a	64.9±3.5^a	65.5±2.6^a	66.0±3.0^a
	10	65.5±2.6	31.4±3.7^c	32.8±3.3^c	40.2±3.7	56.8±2.6^a	68.8±2.8^a
	20	65.3±3.1	18.3±4.6^c	20.7±5.6^c	22.8±2.7^c	40.0±3.5^c	63.1±1.8^a
TMVA	0	69.5±2.7	68.9±1.9^a	69.5±1.5^a	68.3±3.6^a	67.8±4.3^a	68.5±2.7^a
	10	70.7±3.2	32.7±4.6^a	32.6±2.8^c	42.5±3.1^c	59.0±1.2^a	69.8±2.6^a
	20	71.2±2.4	15.3±4.5^c	21.6±5.3^c	40.9±6.7^c	47.7±3.1^b	68.8±1.7^a

Tab 2. Effects of protopine on release of 5-HT from platelets induced by ADP, collagen, AA, or TMVA *in vitro*. $n=6$ rabbits, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Protopine/ μmol·L^{-1}	5-HT in platelet-rich plasma/μg·ml^{-1} ADP	collagen	AA	TMVA
0	0.05±0.03	1.73±0.17	1.82±0.13	2.03±0.14
1	0.04±0.03^a	1.66±0.13^a	1.72±0.11^a	1.89±0.17^a
3	0.05±0.04^a	1.40±0.11^c	1.15±0.13^c	1.66±0.22^a
10	0.03±0.03^a	1.05±0.08^c	1.13±0.15^c	1.42±0.17
30	0.04±0.03^a	0.70±0.17^c	0.89±0.05^c	0.98±0.20
100	0.05±0.03^c	0.38±0.05^c	0.43±0.10^c	0.55±0.21^c
300	0.03±0.03^a	0.08±0.03^c	0.11±0.07^c	0.18±0.12^c
1 000	0.03±0.03^a	0.02±0.03^c	0.01±0.03^c	0.04±0.01^c

Tab 3. Effects of protopine and imidazole on rabbit platelet aggregation and thromboxane B_2 formation induced by AA. $n=6$ rabbits, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline.

Drug/ mmol·L^{-1}	Platelet aggregation/%	Thromboxane B_2/ ng·ml^{-1}
Saline	71.9±3.9	1 481±59
Protopine 1	0.0±0.0^c	1 403±47^a
Imidazole 30	9.2±3.7^c	0.8±0.3^c

Tab 4. Effects of protopine on cAMP and cGMP contents in platelets. $n=6$ rabbits, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* saline.

Drug/ mmol·L^{-1}	cAMP/ nmol/g protein	cGMP/ nmol/g protein
Saline	10.6±3.1	1.7±0.4
Protopine 1	10.6±2.9^a	5.1±1.8^a
Theophylline 4	19.3±5.5^b	
Sodium nitroprusside 0.3		7.7±2.5^c

DISCUSSION

本文选用 ADP，胶原，AA 和 TMVA 为诱导剂分别代表血小板活化的三条途经，即 ADP 途经，AA 代谢途经和血小板活化因子 (platelet activating factor，PAF) 途经(文献报道：TMVA 对血小板的活化作用与 PAF 相

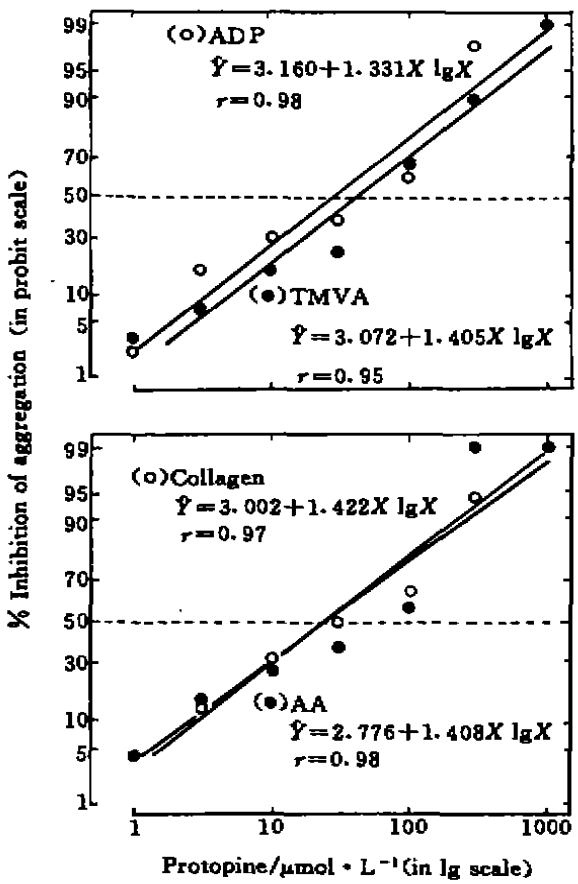

Fig 1. Effects of proteopine on platelet aggregation induced by ADP, collagen, arachidonic acid (AA, and the aggregoserpention of *Trimeresurus mucrosqamatus* venom (TMVA) *in vitro*. n=6 rabbits. $\bar{x}\pm s$.

似，故本文以 TMVA 代替 PAF）观察 Pro 对该三条途经的影响，结果表明：Pro 体内外均能抑制上述四种诱导剂诱导的血小板聚集，提示 Pro 对血小板三条活化途径的抑制无明显选择性.

血小板的活化聚集受血小板内 cAMP 及 cGMP 水平的调节和控制，血小板内 cAMP 及 cGMP 水平升高均可抑制血小板聚集[10]. Pro 能明显升高血小板内 cGMP 水平而不影响 cAMP 水平，说明 Pro 的抗血小板作用与升高血小板内 cGMP 有关. 文献[11]报道：血小板内 cGMP 水平升高可抑制 TXA_2 的生物活性. Pro 能抑制 AA 诱导的血小板聚集而不影响 TXA_2 的生成有可能是由于 Pro 升高血小板内 cGMP 水平，拮抗 TXA_2 对血小板的活化作用而抑制血小板聚集.

血小板活化时释放的许多活性物质可以正反馈地促进血小板聚集. Pro 在抑制胶原、AA 和 TMVA 诱导家兔血小板聚集时也抑制5-HT 释放，表明 Pro 的抗血小板聚集作用也与抑制血小板释放活性物质有关. 以 ADP 为诱导剂未观察到家兔血小板5-HT 释放，与文献[12]报道相符，说明血小板活性物质的释放存在种属差异.

综上所述，Pro 是一血小板功能抑制剂，其对血小板三条活化途经的抑制无明显选择性，作用机理与升高血小板内 cGMP 水平和抑制血小板释放活性物质有关.

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蛇床子总香豆素对类固醇性骨质疏松的作用

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Effect of total coumarins from dried fruits of *Cnidium monnieri* on glucocorticoid-induced osteoporosis in rats

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ABSTRACT Twenty-four 3-month-old ♂ SD rats were divided into 3 groups: Group 1 — control (ig water). Group 2 — ig prednisone 45 $\mu g \cdot kg^{-1}$ twice a week. Group 3 — treated with total coumarins of dried fruits of *Cnidium monnieri* (L) Cuss (TCCM) and prednisone. TCCM was given ig 5 $g \cdot kg^{-1}$, 6 days per week. After 90 d, rats were killed. The proximal tibiae of rats were processed undecalcified for histomorphometric analysis. In comparison with control rats, the bone resorption was enhanced and bone formation decreased. The trabecular bone areas were characterized by reduction of 40 % in rats which received prednisone. Trabecular bone areas of rats treated with TCCM increased 56 % compared with rats receiving prednisone. All indices of bone histomorphometry were near to those in the control. The results showed that TCCM prevented glucocorticoid-induced osteoporosis.

KEY WORDS coumarins; prednisone; osteoporosis; *Cnidium monnieri*

A **摘要** 蛇床子总香豆素（TCCM）5 $g \cdot kg^{-1}$ ig，每周6次，泼尼松45 $\mu g \cdot kg^{-1}$ ig，每周2次，持续90 d. 不脱钙骨制片测量. 结果：泼尼松组大鼠胫骨骨小梁骨吸收增加、骨形成减少、骨小梁面积减少40 %. TCCM加泼尼松组骨小梁面积增加56 %，而各项骨计量学指标接近对照组. 提示TCCM能防治泼尼松引起的骨质疏松.

关键词 香豆素类；泼尼松；骨质疏松症；蛇床

需要长期用糖皮质激素（glucocorticoids，GC）治疗的病人中，约有半数病人发生骨质疏

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