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培垛普利和依那普利拉对大鼠胸主动脉内皮依赖性影响[1]

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关键词 培垛普利; 依那普利拉; 内皮获得性释放因子; 胸主动脉

目的: 研究血管紧张素转换酶(ACE)抑制剂培垛普利(Per)和依那普利拉(Ena)对大鼠内皮细胞的影响 **方法**: 用 Per (2 mg·kg^{-1}·d^{-1})或安慰剂给雄性大鼠(n = 18)灌胃 6 周, 分离主动脉 Ena (0.1 μmol·L^{-1})培养 30 分钟, 离体保存内皮和去内皮的胸主动脉 观察对乙酰胆碱、五羟色胺、苯肾上腺素、硝普钠和硝酸甘油的反应 **结果**: 口服 Per 的胸主动脉与对照组相比增强了乙酰胆碱所引起的内皮依赖性松弛作用, IC_{50}值从 3.84 μmol·L^{-1}(对照组)降到 0.98 μmol·L^{-1}(Per 组) 最大扩张值由 62 ± 9 % 增加到 78 ± 10 % (P < 0.01) 受内皮舒张因子的影响, 五羟色胺和苯肾上腺素引起的收缩反应受抑制, 对照组 EC_{50}值为 6.1 nmol·L^{-1}, 用药组为 8.3 nmol·L^{-1}, 最大收缩值由 2.42 ± 0.29 g (对照组)降到 1.96 ± 0.25 g (处理组) (P < 0.01). 在 Ena 处理的主动脉环上得到同样结果 **结论**: ACE 抑制剂 Ena 和 Per 增加血管内皮依赖性舒张因子的基础释放

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Inhibitory effects of *Acanthopanax gracilistylus* saponins on human platelet aggregation and platelet factor 4 liberation *in vitro*[1]

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KEY WORDS *Acanthopanax gracilistylus*; saponins; platelet aggregation; platelet factor 4; thrombosis

AIM: To study the effects of *Acanthopanax gracilistylus* var *pubescens* Li saponins (AGVPS) on human platelet aggregation and platelet factor 4 (PF4) *liberation in vitro*. **METHODS**: Human platelet aggregations induced by ADP, adrenaline, and collagen were measured turbidimetrically. The aggregation curve was recorded on a platelet aggregometer and the maximal aggregation rate (AR_{max}), effective deaggregation rate in 5 min ($DR_{5\ min}$) and lag time (LT) were autocalculated by the built-in microcomputer; PF4 liberation from human platelets stimulated by ADP and collagen was determined by recording the heparin thrombin clotting time (HTCT). Thrombosis was tested by weighing the wet and dry thrombi formed in a siliconized revolving ring. **RESULTS**: AGVPS inhibited *in vitro* the AR_{max} with IC_{50} of 1.33 (95 % confidence limits: 1.09 − 1.63, ADP-induced), 1.66 (1.54 − 1.79, adrenaline-induced), and 4.2 g·L^{-1} (0.6 − 29, collagen-induced). The $DR_{5\ min}$ (on ADP-induced aggregation) and LT (collagen-induced) were also increased as well. Meanwhile, AGVPS 0.63 − 2.50 g·L^{-1} prolonged HTCT on ADP- and collagen-stimulated PF4 liberation At 0.34 − 1.39 g·L^{-1}, AGVPS reduced the wet and dry weight of thrombi formed *in vitro*. **CONCLUSION**: AGVPS inhibits human platelet aggregation, liberation, and thrombosis *in*

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vitro, suggesting its possible antithrombotic action in man.

The total saponins extracted from *Acanthopanax gracilistylus* var *pubescens* (pampanin) Li (AGVPS), showed protective effects on myocardial ischemia[1], hypercholesterolemia[2], and beneficial effects against arrhythmias induced by chloroform, $BaCl_2$, isoprenaline, and ouabaine[3] as well as by ischemia and reperfusion[4]. The platelet plays an important role in the development of coronary heart diseases and arrhythmias[5-6]. Our present work was undertaken to study the effects of AGVPS on human platelet aggregation, Platelet factor 4 (PF4) liberation, and thrombosis *in vitro*.

MATERIALS AND METHODS

Drugs and reagents AGVPS, a yellowish crystal powder, was extracted from the Chinese herb *Acanthopanax gracilistylus* var *pubescens* with classical method[7] and determined in Guangzhou Institute of Medical and Pharmaceutical Industry. The saponin content of AGVPS was 28.9 % determined with photocolorimetric method. Ten spots of ginsenosides were shown on the silica gel thin-layer plate, four of them having the same R_f values as Rb_1, R_d, R_e, and R_g components of ginsenosides (Laboratory data of Hunan Medical University). It was dissolved in normal saline and the preparations were freshly made before each use. ADP was purchased from Fluka Chemical Co, Switzerland, lot № 263138-387. Adrenaline (lot № 910118) and thrombin (lot № 920812) were produced by Guangzhou Minxin and Zhuhai Biochemical Pharmaceutic Factories, respectively. They were dissolved in 0.2 mol·L^{-1} phosphate buffer solution (PBS, pH 7.2) and stocked at −20 ℃. Collagen was made from rabbit tendon[8] and stored at 4 ℃ for <4 d.

Platelet aggregation test Venous blood was obtained by vein puncture from healthy college students of either sex, aged 21 ± 2 a, who had taken neither medicine nor greasy food for at least 2 wk. The blood samples were anticoagulated with 3.4 % sodium citrate (9:1, vol/vol) and centrifuged at 190 × g for 5 min to get platelet-rich plasma (PRP). The precipitate was centrifuged at 1800 × g for 10 min to obtain the platelet-poor plasma (PPP). The platelet count in each PRP was adjusted to 2.5×10^{11} · L^{-1} by dilution with PPP from the same sample. PRP 0.2 mL was placed in a curvette and stirred with AGVPS or control solution 25 μL at 37 ℃ for 5 min, then aggregating agent 25 μL was added (final concentration: ADP 4.7 μmol · L^{-1}, adrenaline 6.2 μmol · L^{-1} and collagen 40 mg·L^{-1}). The platelet aggregation was measured with a platelet aggregometer (PLA-4, Shanghai, China), and the maximal aggregation rate (AR_{max}), effective deaggregation rate in 5 min ($DR_{5\ min}$) and lag time (LT)[9,10] were autocalculated by the built-in microcomputer.

PF4 liberation The biological activity of PF4 was determined by evaluating the antiheparin activity in a thrombin clotting time[11]. The platelet counts were adjusted to $(2.5 \pm 0.5) \times 10^{11} \cdot L^{-1}$. AGVPS or control solution 50 μL was added to PRP 0.4 mL. It was incubated at 37 ℃ and stirred with a magnetic bar at 1000 rpm. Five min after 50 μL ADP (4.7 μmol·L^{-1}) or collagen (40 mg·L^{-1}) was added, the reaction was stopped in ice bath. The mixture was centrifuged at 1700 × g for 10 min to get a supernatant of PPP. To PPP 0.1 mL, heparin (100 IU·L^{-1}) 0.1 mL was added, and the heparin thrombin clotting time (HTCT) was recorded at 37 ℃

Thrombosis experiment[12,13] Venous blood 1.6 mL was injected into a siliconized revolving ring containing AGVPS or control solution 0.2 mL. The ring rotated on the thrombosis apparatus (XSN-Ⅲ, Wuxi, China) at a speed of 18 cycles per minute for 15 min. The thrombus formed in the ring was taken out and its wet weight was measured. It was dried at 65 ℃ for 20 min to get the dry weight.

RESULTS

Effect on platelet aggregation AGVPS resulted in concentration-dependent inhibition of platelet aggregation induced by ADP, adrenaline, and collagen. The IC_{50} values were 1.33 (95 % confidence limits: 1.09 − 1.63), 1.66 (1.54 − 1.79), and 4.2 (0.6 − 29) g·L^{-1}, respectively. AGVPS also increased $DR_{5\ min}$ on ADP-induced aggregation in a concentration-dependent way. However, on adrenaline- and collagen-induced aggregation, $DR_{5\ min}$ was little affected by AGVPS (Tab 1).

Effect on PF4 liberation AGVPS prolonged HTCT, showing it suppressed the PF4 liberation stimulated by ADP and collagen (Tab 2).

Effect on human thrombosis Both the wet and dry thrombus weights were markedly reduced by AGVPS at 0.34 − 1.39 g·L^{-1} (Tab 3).

DISCUSSION

Acanthopanax gracilistylus var *pubescens* and *Panax ginseng* are in the same *Araliaceae* family. The main components of AGVPS have been identified as Rb_1, R_g, R_d, and R_e components of

Tab 1. Effect of AGVPS on platelet aggregation induced by ADP, adrenaline, and collagen. AR_{max}: maximal aggregation rate; IR: inhibition rate; $DR_{5\ min}$: effective deaggregation rate in 5 min; LT: lag time. $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control.

Drug/g·L^{-1}		AR_{max}/%	IR/%	$DR_{5\ min}$/%	LT/s
Collagen-induced ($n=5$)					
Control	0	62 ± 7		3.0 ± 1.9	54 ± 9
AGVPS	0.63	55 ± 8^b	11.3	2.2 ± 0.5^a	63 ± 11^b
	1.25	54 ± 7^c	12.9	2.9 ± 2.3^a	77 ± 9^b
	2.50	37 ± 11^c	40.3	3.2 ± 2.7^a	127 ± 45^c
ADP-induced ($n=5$)					
Control	0	68 ± 7		0.5 ± 0.6	
AGVPS	0.63	55 ± 4^b	19.1	7.3 ± 4.9^b	
	1.25	32 ± 9^c	52.9	17.3 ± 11.2^c	
	2.50	18 ± 8^c	73.5	60.6 ± 42.3^c	
	5.0	6 ± 2^c	90.9	100.0 ± 0^c	
Adrenaline-induced ($n=6$)					
Control	0	57 ± 10		0.9 ± 1.2	
AGVPS	0.63	51 ± 11^a	10.5	0.7 ± 0.7^a	
	1.25	38 ± 10^c	33.3	1.6 ± 1.9^a	
	2.50	16 ± 7^c	71.9	27.3 ± 31.4^c	

Tab 2. Effect of AGVPS on PF4 liberation stimulated by ADP and collagen. $\bar{x} \pm s$. $^bP<0.05$, $^cP<0.01$ vs control.

AGVPS/g·L^{-1}	Heparin thrombin clotting time/s	
	ADP ($n=6$)	Collagen ($n=8$)
0	20.1 ± 1.8	31.5 ± 9.6
0.63	24.1 ± 3.6^b	42.4 ± 12.8^b
1.25	26.3 ± 3.1^b	42.8 ± 9.3^b
2.50	27.5 ± 3.1^c	43.8 ± 10.1^b

Tab 3. Effect of AGVPS on human thrombosis *in vitro*. $n=5$, $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ vs control.

AGVPS/g·L^{-1}	Thrombus weight/mg	
	Wet	Dry
0	52.5 ± 12.3	18.9 ± 6.3
0.34	39.1 ± 8.6^b	14.5 ± 2.6^b
0.70	43.7 ± 5.9^b	15.9 ± 3.4^b
1.39	38.1 ± 4.0^c	13.8 ± 2.2^c

ginsenosides. Ginsenosides inhibited rabbit platelet aggregation with IC_{50} of 0.92 (AA-induced), 2.10 (ADP-induced) and 2.70 g · L^{-1} (collagen-induced)[14]. The IC_{50} values of AGVPS on human platelet aggregation induced by ADP, adrenaline, and collagen were 1.33, 1.66, and 4.2 g·L^{-1}, respectively, suggesting its potent antiplatelet actions. PF4 is a platelet-specific protein stored in α granules of platelets. The PF4 release represents platelet liberation to some extent. Platelet aggregation and liberation are the indicators of platelet activation, which play an important role in thrombosis. In this study, AG was proved to have marked inhibition of human thrombosis *in vitro*. This might be due to its suppressing platelet aggregation and liberation, ie, inhibiting the platelet activation. Besides, during the activation, other platelet-specific proteins such as β-thromboglobin (β-TG) and platelet-derived growth factor (PDGF) stored in α granules of platelet can be released together with PF4, suggesting that AGVPS may also inhibit the β-TG and PDGF liberation[6]. PDGF is an important induer of atherosclerosis, and β-TG has potent inhibition of PGI_2 in vessel walls. PF4, β-TG, and PDGF, as well as platelet aggregation and thrombosis, play important roles in atherosclerosis[5,6]. Thus, the beneficial effects of AGVPS on hypercholesterolemia[2] and atherosclerosis (to be published) may result from the inhibitory effects of AGVPS on platelet aggregation, liberation and thrombosis. In addition, since many cardiovascular diseases are closely related to platelet function, the inhibitory effects of AGVPS on platelet activation may have an important part in its beneficial effects on myocardial infarction and cardiac arrhythmias[1,4]. In conclusion, AGVPS inhibited human platelet aggregation, liberation and thrombosis. It may be a promising new medicine from traditional herb.

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短毛五加总皂苷在体外抑制人血小板聚集和血小板因子 4 释放[1]

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关键词 短毛五加；皂苷类；血小板聚集；血小板因子 4；血栓形成

目的：研究短毛五加总皂苷(AGVPS)在体外对人血小板聚集和血小板因子 4 (PF4)释放反应的影响. **方法**：采用比浊法测定人血小板聚集，肝素凝血酶凝集时间(HTCT)法测定人 PF4 释放反应 **结果**：AGVPS 浓度依赖性地抑制血小板 AR_{max}，其 IC_{50} 分别为 1 33 (ADP 诱导)，1 66 (肾上腺素诱导)和 4.2 $g \cdot L^{-1}$ (胶原诱导) 同时提高 $DR_{5\ min}$ (ADP 诱导)及延长诱导迟缓期(胶原诱导)；在 ADP 和胶原刺激的人 PF4 释放反应中，AGVPS 延长其 HTCT. 同时，AGVPS 减轻体外形成血栓的干、湿重. **结论**：AGVPS 在体外对人血小板聚集和释放反应及血栓形成具有显著的抑制作用

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