

Endothelium-dependent effect of perindopril and enalaprilat in rat thoracic aorta¹

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KEY WORDS perindopril; enalaprilat; endothelium-derived relaxing factor; thoracic aorta

AIM: To study the effect of the angiotensin-converting enzyme (ACE) inhibitors perindopril (Per) and enalaprilat (Ena) on the reactivity of the endothelium in normal rats. **METHODS:** Male rats were treated intragastrically with Per (2 mg·kg⁻¹·d⁻¹) or placebo (n = 18) for 6 wk. Aorta was isolated for experiment. Another set of isolated aortic rings with and without endothelium were incubated with Ena (0.1 μmol·L⁻¹) for 30 min. Responses to acetylcholine, serotonin, phenylephrine, sodium nitroprusside (SN), and nitroglycerin (Nit) were observed. **RESULTS:** Endothelium-dependent relaxation to acetylcholine was augmented in aortic rings from rats treated with Per in comparison with control. The IC₅₀ value (95 % confidence limits) decreased from 3.8 (0.56 - 26.1) μmol·L⁻¹ (control group) to 0.98 (0.28 - 3.41) μmol·L⁻¹ (Per-treated group). The maximal relaxation was augmented from 62 ± 9 % to 78 ± 10 % (P < 0.01). However, the responses to the endothelium-independent vasodilators, SN and Nit, were similar. Serotonin- and phenylephrine-induced contractions were decreased, which were influenced by basal release of endothelium-derived relaxing factor (EDRF). EC₅₀ value was 6.1 (2.6 - 14.4) nmol·L⁻¹ vs 8.3 (3.6 - 18.8) nmol·L⁻¹ in comparison with control group and Per-treated group. The maximal contraction was decreased from 2.42 ± 0.29 g (control group) to 1.96 ± 0.25 g (treated group) (P < 0.01). Similar results were found in incubation with Ena. **CONCLUSION:** Ena and Per enhanced the basic release of EDRF from vascular endothelium.

Angiotensin-converting enzyme (ACE), located mainly at the cell membrane of the endothelial cells, converts the less-active peptide angiotensin I into the powerful vasoconstrictor angiotensin II^[1], which acts both as a direct activator of vascular smooth muscle and as an amplifier of the sympathetic nervous system. Inhibitors of ACE cause peripheral vasodilatation by reducing the local and circulating level of angiotensin. ACE inhibition decreased the breakdown of bradykinin^[2,3] which dilates vessels by releasing endothelium-derived relaxing factor (EDRF) identified as nitric oxide (NO) as well as a putative hyperpolarizing factor from endothelial cells^[4,5] and by inhibiting the release of endothelin^[6,7].

Sulfhydryl-containing ACE inhibitor captopril improves endothelial function^[8,9], but the effects of non-sulfhydryl containing ACE inhibitors on endothelium in normal vessels were little known. The goal of the present study was to investigate whether nonsulfhydryl long-lasting ACE inhibitors enalaprilat (Ena) and perindopril (Per) affect the reactivity of endothelium and smooth muscle to vasoconstrictor and vasodilator stimuli in normal rats.

MATERIALS AND METHODS

Aortic ring Wistar rats (♂, n = 18, weighing 262 ± 27 g) were randomly assigned to 2 groups: 1) given intragastrically (ig) Per 2 mg·kg⁻¹·d⁻¹ in 1 mL of distilled water and 2) given 1 mL of water for 6 wk, 24 h after the last medication, the rats were killed by cervical dislocation under ether anesthesia.

The thoracic aorta was removed and placed in cold Krebs-Henseleit solution. The apparatus and procedure were similar to those reported previously^[10]. To study the effects of vascular ACE activity on vascular relaxation, isolated rat aorta preparations were exposed to acetylcholine (10 nmol·L⁻¹ to 100 μmol·L⁻¹) or nitroglycerin (Nit, 0.1 nmol·L⁻¹ to 1 μmol·L⁻¹). To study the effects of vascular ACE activity on vascular contractile properties, isolated rat aorta rings were

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exposed to phenylephrine ($0.1 \text{ nmol} \cdot \text{L}^{-1}$ to $10 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) and serotonin ($10 \text{ nmol} \cdot \text{L}^{-1}$ to $100 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$).

In vitro treated with Ena, male Wistar rats ($n = 8$) weighing $229 \pm 32 \text{ g}$ were killed by cervical dislocation under ether anesthesia. The following procedure is the same as above. During the dissection the utmost care was taken to protect the endothelial lining. In some preparations, the endothelium was removed by gently rubbing the intimal surface with a forceps. To study the effects of vascular ACE activity on vascular relaxation, isolated rat aorta preparations with and without endothelium were exposed to acetylcholine ($10 \text{ nmol} \cdot \text{L}^{-1}$ to $100 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) or SN ($0.1 \text{ nmol} \cdot \text{L}^{-1}$ to $100 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$). The aortic rings then were incubated with the ACE inhibitor Ena ($0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) for 30 min, and concentration-response curves to acetylcholine or SN were repeated. To study the effects of vascular ACE activity on contractile properties of the blood vessels, experiments were performed in rings with and without endothelium exposed to phenylephrine ($0.1 \text{ nmol} \cdot \text{L}^{-1}$ to $10 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) and serotonin ($10 \text{ nmol} \cdot \text{L}^{-1}$ to $100 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$). The rings then were incubated with Ena ($0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) for 30 min, and concentration response curves to phenylephrine and serotonin were repeated. All experiments were done in the presence of indometacin ($10 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) to avoid any prostaglandin production.

Drug Per (Les Laboratoires Servier, Gidy-45400 Fleury-Les-Aubrais, France), acetylcholine, phenylephrine, serotonin, indometacin (Sigma, USA), SN (Wu Han Pharmaceutical Factory), Nit (Beijing Yi Ming Pharmaceutical Factory). Acetylcholin and Nit were dissolved in distilled water. Phenylephrine and serotonin were dissolved in water containing 1 % ascorbic acid. Indometacin was dissolved in an equimolar solution of sodium bicarbonate.

Calculation The effective concentration causing 50 % of the maximal relaxation (IC_{50}) and the EC_{50} were defined as the concentrations of agonist inducing a response of 50 % of maximal tension of the agonist, and their 95 % confidence limits were calculated by Bliss method. In quiescent rings, contractile responses were expressed as contractile tension. IC_{50} and EC_{50} values were expressed as pD_2 . Statistical evaluation was made by paired t test for comparison on the same ring before and after incubation with Ena, by unpaired t test for comparison between Per and control rats.

RESULTS

1 Aorta of rat treated with Per for 6 wk

1.1 Relaxation to acetylcholine The endothelium-dependent relaxation of rat aortic rings was produced by acetylcholine ($10 \text{ nmol} \cdot \text{L}^{-1}$ to $100 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$). Acetylcholine-induced relaxation was potentiated. The IC_{50} value (95 % confidence

limits) decreased from $3.8 (0.56 - 26.1) \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (control group) to $0.98 (0.28 - 3.41) \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ (Per-treated group). The maximal relaxation was augmented from $62 \pm 9 \%$ to $78 \pm 10 \%$ ($n = 18$; $P < 0.01$) (Fig 1).

1.2 Contraction to serotonin and phenylephrine Serotonin ($10 \text{ nmol} \cdot \text{L}^{-1}$ to $100 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) induced contractions, which were significantly different from those of rings from control group (Fig 1). EC_{50} value (95 % confidence limits) was $0.38 (0.18 - 0.79) \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ vs $0.52 (0.24 - 1.1) \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ in comparison with control group and Per-treated group, but maximal contraction were decreased from $2.34 \pm 0.28 \text{ g}$ (control group) to $1.84 \pm 0.29 \text{ g}$ (treated group) ($n = 18$; $P < 0.01$).

Likewise, the contractions induced with phenylephrine ($0.1 \text{ nmol} \cdot \text{L}^{-1}$ to $10 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) were also different in the rings from Per-treated and control rats (Fig 1). EC_{50} value (95 % confidence limits) was $6.1 (2.6 - 14.4) \text{ nmol} \cdot \text{L}^{-1}$ vs $8.3 (3.6 - 18.8) \text{ nmol} \cdot \text{L}^{-1}$ in comparison with control group and Per-treated group. The maximal contraction was decreased from $2.42 \pm 0.29 \text{ g}$ (control group) to $1.96 \pm 0.25 \text{ g}$ (treated group) ($n = 18$; $P < 0.01$).

1.3 Relaxation to Nit In rat aortic rings precontracted with phenylephrine ($0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) the responses to Nit were similar in the rings from Per-treated and control groups (Fig 1).

2 Aorta treated *in vitro* with Ena The experiments were performed in adjacent rings with and without endothelium of the same aorta.

2.1 Effect on acetylcholine-induced relaxation Acetylcholine produced dose-dependent relaxations of rings with endothelium from normotensive rats. After incubation with Ena $0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ for 30 min, acetylcholine-induced relaxation was potentiated in the same rings. The IC_{50} value (95 % confidence limits) decreased from $1.1 (0.12 - 9.6) \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ to $76.4 (5.4 - 1100) \text{ nmol} \cdot \text{L}^{-1}$ (Fig 1).

2.2 Effect on serotonin-induced contraction In unrubbed rings, incubation with Ena $0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ decreased the maximal response to serotonin ($n = 8$; $P < 0.01$) (Fig 1). On the contrary, the response after the second concentration of

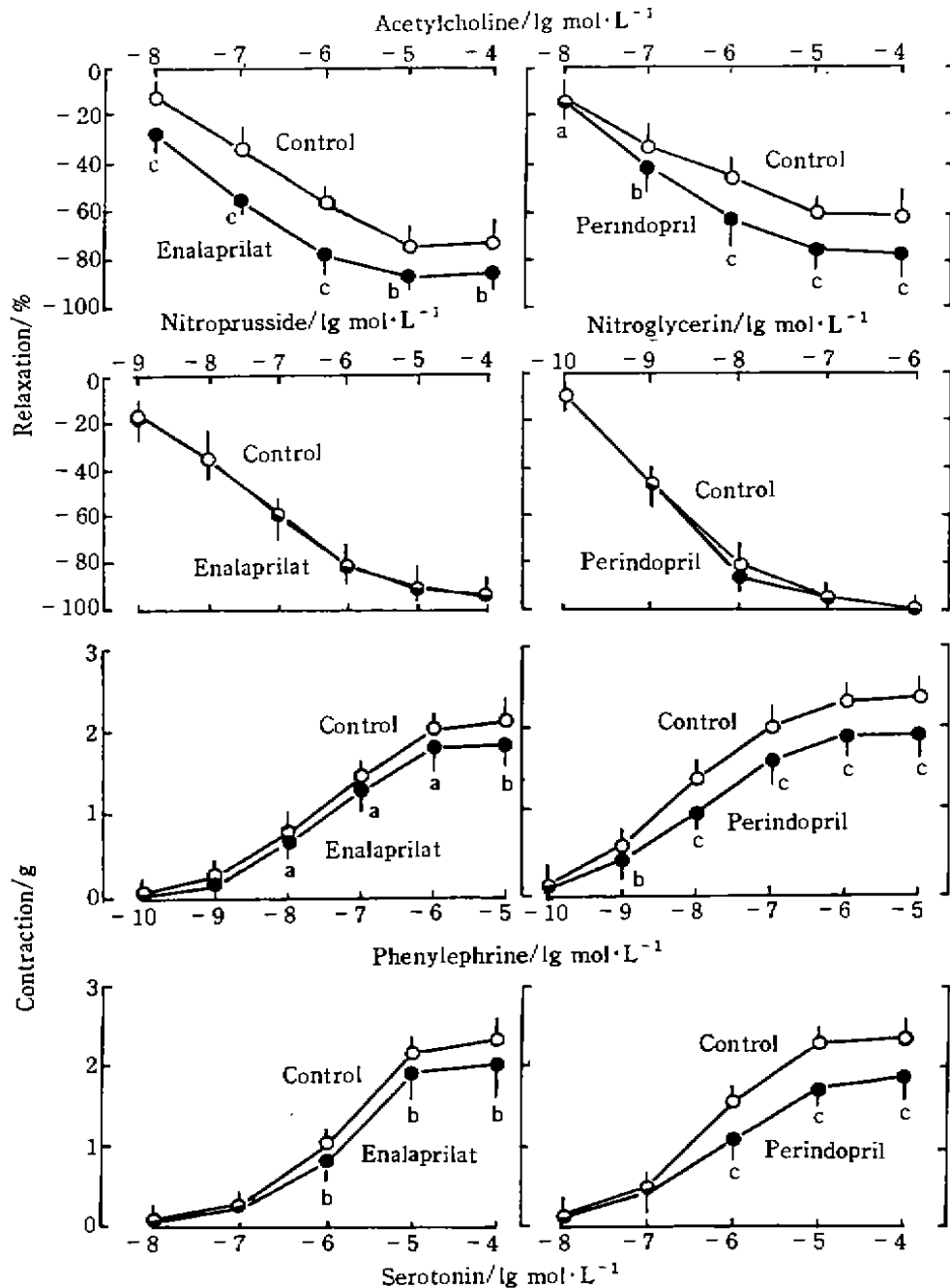


Fig 1. Relaxation and contraction in rat aorta isolated from *in vitro* enalaprilat (Ena) incubation, *in vivo* perindopril (Per) treatment and control. * $P > 0.05$, $^bP < 0.05$, $^cP < 0.01$. For relaxation in response to nitroprusside and nitroglycerin, *in vitro* Ena-treated and control rings were precontracted with KCl ($20 \text{ mmol} \cdot \text{L}^{-1}$), while *in vivo* Per treatment and control rat aorta were precontracted with phenylephrine ($0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$).

serotonin was similar to the first one in rings that had not been incubated with Ena. Incubation with Ena had no effect on the resting tone.

In rubbed preparations, incubation with Ena had no change on the response for serotonin.

2.3 Effect on phenylephrine-induced con-

traction In the rings with endothelium, Ena ($0.1 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$) incubation decreased the response to phenylephrine ($n = 8$; $P < 0.05$) (Fig 1). On the contrary, the response to the second concentration of phenylephrine was similar to the first one in rings that had not been incubated with Ena. In the rings

without endothelium, incubation with Ena ($0.1 \mu\text{mol} \cdot \text{L}^{-1}$) gave no change on the dose-response curves for phenylephrine.

2.4 Effect on SN-induced relaxation In KCl $20 \text{ mmol} \cdot \text{L}^{-1}$ precontracted rings with or without endothelium, incubation with Ena ($0.1 \mu\text{mol} \cdot \text{L}^{-1}$) for 30 min did not modify the response for SN: the IC_{50} value (95 % confidence limits) was $50.0 (8.0 - 310) \text{ nmol} \cdot \text{L}^{-1}$ vs $47.4 (7.4 - 300) \text{ nmol} \cdot \text{L}^{-1}$ in comparison with control and Ena ($0.1 \mu\text{mol} \cdot \text{L}^{-1}$)-incubated rings with endothelium and $53.1 (10.1 - 280) \text{ nmol} \cdot \text{L}^{-1}$ vs $35.1 (6.4 - 190) \text{ nmol} \cdot \text{L}^{-1}$ in rubbed control compared with Ena-incubated rings.

DISCUSSION

In this experiment, our results showed that Per *in vivo* treatment and in isolated rat aorta with intact endothelium treated with Ena augmented relaxation to acetylcholine, decreased the contraction to serotonin and phenylephrine, but did not change the relaxation induced with Nit and SN. However, in rubbed endothelial aortic rings, Ena did not influence the response to acetylcholine, serotonin and phenylephrine. It was well known that the relaxation induced by acetylcholine is dependent on the presence of vascular endothelium^[4] and contractile responses to serotonin and phenylephrine in rat aorta were depressed by spontaneously released EDRF, which was considered as an endogenous functional antagonist opposing the activation of contractors^[11], but Nit and SN were endothelium-independent vasodilators. Our study suggested that ACE inhibitors Per and Ena *in vivo* and *in vitro* treatment induced the change in the production or release of endothelial relaxing factors rather than an alteration of sensitivity of vascular smooth muscle.

The mechanism of endothelium-dependent effects of ACE inhibitors is not well known. Recently it is demonstrated that ACE inhibitors affect vascular response by at least both endothelium-dependent and bradykinin-mediated mechanism^[12]. ACE inhibitors protect against the breakdown of bradykinin. Bradykinin stimulates the release of endothelium-derived vasodilator mediators, including NO, endothelium-derived hyperpolarizing factor and

prostacyclin. However, ACE inhibitors evoke endothelium-dependent relaxation in arteries stimulated with threshold concentration of bradykinin, which cannot be attributed to an inhibitors of bradykinin degradation^[12,13].

In conclusion, this study suggests that the nonsulfhydryl-containing ACE inhibitors, Per and Ena *in vivo* and *in vitro* treatment may increase the production and release of EDRF from endothelial cells.

REFERENCES

- 1 Caldwell PR, Seegal BC, Hsu KC, Das M, Sofier RL. Angiotensin-converting enzyme: vascular endothelial localization. *Science* 1976; **191**: 1050-1.
- 2 Vanhoutte PM, Auch-Schwelk W, Biondi ML, Lorenz RR, Schini VB, Vidal MJ. Why are converting enzyme inhibitors vasodilators? *Br J Clin Pharmacol* 1989; **28** (Suppl 2): 95S-104S.
- 3 Wiemer G, Schwilke BA, Becker RH, Busse R. Ramiprilat enhances endothelial nitric oxide formation by inhibiting breakdown of endothelium-derived bradykinin. *Hypertension* 1991; **18**: 558-63.
- 4 Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980; **288**: 373-6.
- 5 Taylor SG, Weston AH. Endothelium-derived hyperpolarizing factor: a new endogenous inhibitor from the vascular endothelium. *Trends Pharmacol Sci* 1988; **9**: 272-4.
- 6 Yoshida H, Nakamura M. Inhibition by angiotensin converting enzyme inhibitors of endothelin secretion from cultured human endothelial cells. *Life Sci* 1992; **50**: PL195-200.
- 7 Momose N, Fukuo K, Morimoto S, Ogihara T. Captopril inhibits endothelin-1 secretion from endothelial cells through bradykinin. *Hypertension* 1993; **21**: 921-4.
- 8 van Gilst WH, de Graeff PA, de Leeuw MJ, Scholten E, Wesseling H. Converting enzyme inhibitors and the role of the sulfhydryl group in the potentiation of exo- and endogenous nitrovasodilators. *J Cardiovasc Pharmacol* 1991; **18**: 429-36.
- 9 Clozel M. Mechanism of action of angiotensin converting enzyme inhibitors on endothelial function in hypertension. *Hypertension* 1991; **18** (4 Suppl): 1137-42.
- 10 Berkenboom G, Fang ZY, Unger P, Fontaine J. Effects of *in vivo* SIN1 treatment on nitrovasodilator relaxation and on EDRF-mediated responses in rat aorta. *J Cardiovasc Pharmacol* 1990; **16**: 636-40.
- 11 Martin W, Furchgott RF, Villani GM, Jothanandan D. Depression of contractile responses in rat aorta by spontaneously released endothelium-derived relaxing factor. *J Pharmacol Exp Ther* 1986; **237**: 529-38.
- 12 Bossaller C, Auch-Schwelk W, Grafe M, Graf K, Baumgarten C, Fleck E. Effects of converting enzyme inhibition on endothelial bradykinin metabolism and endothelium-dependent vascular relaxation

Agents Actions Suppl 1992; 38 (Pt 3): 171-7

- 13 Vanhoutte PM, Boulanger CM, Illiano SC, Nagao T, Vidal M, Mombouli JV.

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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

R 972

R P 15.1

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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,分离主动脉内皮(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,IC₅₀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EC₅₀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 IC₅₀ 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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