

Platelet activating factor-induced P-selectin expression in platelets and its related signal transduction¹

ZENG Shan, Yi Fu-Xian, GUO Zhao-Gui²

(Laboratory of Molecular Pharmacology, Hunan Medical University, Changshu 410078, China)

KEY WORDS platelet activating factor; P-selectin; amiloride; genistein; egtazic acid; flow cytometry; blood platelets

ABSTRACT

AIM: To study the intracellular signal transduction mechanisms of platelet activating factor (PAF)-induced platelet P-selectin expression. **METHODS:** Human blood platelets were used to test the effect of PAF-induced P-selectin expression using flow cytometry. **RESULTS:** PAF 20 nmol·L⁻¹ elicited a moderate up-regulation of P-selectin expression [(47.5 ± 1.3) % vs control (3.8 ± 0.9) %, *P* < 0.01]. Pretreatment with egtazic acid (EGTA) 2 mmol·L⁻¹ and 5, 5'-dimethyl-bis-(*o*-aminophenoxy)-ethane-*N, N, N', N'*-tetracetic acid (BAPTA) 200 μmol·L⁻¹ to block Ca²⁺ influx or chelate the intracellular calcium, respectively, reduced P-selectin expression in response to PAF [(13.3 ± 0.9) % and (16.8 ± 1.9) % vs (47.5 ± 1.3) % of PAF group, *P* < 0.01]. Inhibition of Na⁺/H⁺ exchange with amiloride (Ami) 400 μmol·L⁻¹ resulted in an inhibition of P-selectin expression [(37.5 ± 2.1) % vs (47.5 ± 1.3) % of PAF group, *P* < 0.01]. Genistein (Gen) 300 μmol·L⁻¹ to inhibit protein tyrosine phosphorylation showed similar effect [(29 ± 4) % vs (47.5 ± 1.3) % of PAF group, *P* < 0.01]. **CONCLUSION:** Multiple signal transduction pathways, including protein tyrosine phosphorylation, Na⁺/H⁺ exchange, and Ca²⁺ mobilization, mediated PAF-induced P-selectin expression.

INTRODUCTION

Adhesion molecules, such as P-selectin, are potential markers for evaluating platelet activation and studying the role of cell-cell interactions in numerous biological processes related to hemostasis and inflammation^[1,2]. Surface-expressed P-selectin mediates the binding of activated platelets to neutrophils and monocytes involved in early inflammatory reactions^[3,4]. There is also growing interest in role of α-granule release in modulation of vascular injury and repair mechanisms^[5]. While the regulatory mechanism for P-selectin expression in α-granule of activated platelets is poorly understood, it apparently involves a complex sequence of activation processes including structural transformation and translocation of α-granules and secretion^[6]. This study aims to investigate the intracellular signal transduction pathways, including Na⁺/H⁺ exchange, protein tyrosine phosphorylation, and Ca²⁺ mobilization, on the secretion of α-granules and P-selectin expression in response to platelet activating factor (PAF).

MATERIALS AND METHODS

Drugs and reagents PAF, amiloride (Ami), genistein (Gen), [5, 5'-dimethyl-bis-(*o*-aminophenoxy)-ethane-*N, N, N', N'*-tetracetic acid] (BAPTA), egtazic acid (EGTA), indomethacin, bovine serum albumin (BSA), edetic acid (EDTA) were purchased from Sigma Chemical Co. Mouse monoclonal antibodies against human P-selectin (CD62, GMP-140, or PADGEM) was from Suzhou Medical College. The secondary antibody (goat anti-mouse IgG-phycoerythrin conjugate) was from Huamei Biotechnology Inc.

Platelet preparation and stimulation 20 healthy volunteers (12 male, 8 female), aged 28 a ± s 4 a, and weighing 52 kg ± s 7 kg, have not received

¹ Project supported by the National Natural Science Foundation of China, No 39570816.

² Correspondence to Prof GUO Zhao-Gui.

Phn 86-731-447-4411, ext 2797. Fax 86-731-447-1339.

E-mail guozg@public.cs.hn.cn

Received 1998-07-13

Accepted 1999-03-11

any aspirin for at least 1 a. Platelets were prepared from fresh human blood of these volunteers⁽⁷⁾. The final platelet suspensions contained Ca^{2+} $1 \text{ mmol} \cdot \text{L}^{-1}$, indomethacin $10 \mu\text{mol} \cdot \text{L}^{-1}$ and apyrase $1 \text{ mg} \cdot \text{L}^{-1}$. Platelets were incubated with PAF $20 \text{ nmol} \cdot \text{L}^{-1}$ at $37 \text{ }^\circ\text{C}$ for 3 min without stirring. In other experiments, platelets were treated with Ami $400 \mu\text{mol} \cdot \text{L}^{-1}$, Gen $300 \mu\text{mol} \cdot \text{L}^{-1}$, BAPTA $200 \mu\text{mol} \cdot \text{L}^{-1}$ or EGTA $2 \text{ mmol} \cdot \text{L}^{-1}$, respectively, for 10 min before PAF stimulation. After incubation, platelets were fixed by 1 % paraformaldehyde in phosphate buffer solution (PBS) at pH 7.4 at $4 \text{ }^\circ\text{C}$ for 60 min and washed twice with PBS containing 0.02 % BSA, then incubated with the primary anti-P-selectin at $4 \text{ }^\circ\text{C}$ for 30 min. After washed with PBS, platelets was then incubated with the secondary antibody at a 1:100 dilution for 20 min. The stained platelets were washed twice and immediately analyzed by flow cytometry (Coulter Epics).

Flow cytometry The determination of the percentage of platelet expressing P-selectin was carried out⁽⁸⁾. Mean P-selectin fluorescence for the entire platelet population was expressed in arbitrary fluorescence units.

Statistics Results were expressed as $\bar{x} \pm s$ and compared by ANOVA and *t*-test.

RESULTS

Platelet P-selectin expression Preliminary experiments were performed to identify the time course of peak P-selectin expression in response to PAF, the percentage of P-selectin-positive platelets was found to plateau at 3 min after addition of PAF. The baseline percentage of platelet expressing P-selectin in the PRP before PAF or diluent was $(3.8 \pm 0.9) \%$. The P-selectin expression was increased to $(47.5 \pm 1.3) \%$ after PAF stimulation (Tab 1).

Effects of amiloride, genistein, BAPTA and EGTA on P-selectin expression Inhibition of Na^+/H^+ exchange with Ami $400 \mu\text{mol} \cdot \text{L}^{-1}$ suppressed platelet P-selectin expression in response to PAF. Platelet aggregation was also completely inhibited at the same concentration. Gen $300 \mu\text{mol} \cdot \text{L}^{-1}$ showed similar inhibitory effect on P-selectin expression. To determine whether inhibition of intracellular Ca^{2+} elevation and Ca^{2+} entry affected α -granule release,

Tab 1. Effects of various treatments on PAF-stimulated P-selectin expression. $n=4$ independent experiments, $\bar{x} \pm s$. $^{\circ}P < 0.01$ vs control, $^{\text{f}}P < 0.01$ vs PAF.

Treatments	P-Selectin-positive platelet/ %
Basal control	3.8 ± 0.9
PAF $20 \text{ nmol} \cdot \text{L}^{-1}$	$47.5 \pm 1.3^{\circ}$
Ami $400 \mu\text{mol} \cdot \text{L}^{-1}$ + PAF	$37.5 \pm 2.1^{\text{f}}$
Gen $300 \mu\text{mol} \cdot \text{L}^{-1}$ + PAF	$29 \pm 4^{\text{f}}$
BAPTA $200 \mu\text{mol} \cdot \text{L}^{-1}$ + PAF	$16.8 \pm 1.9^{\text{f}}$
EGTA $2 \text{ mmol} \cdot \text{L}^{-1}$ + PAF	$13.3 \pm 0.9^{\text{f}}$

platelets were treated with BAPTA $200 \mu\text{mol} \cdot \text{L}^{-1}$ and EGTA $2 \text{ mmol} \cdot \text{L}^{-1}$, the results showed that the percentage of P-selectin-positive platelets was reduced (Tab 1).

DISCUSSION

Based on our previous study, PAF appears to activate platelets by multiple signal transduction pathways^(9,10). Intracellular Ca^{2+} mobilization and Na^+/H^+ exchange are both early event in PAF-induced activation⁽⁹⁻¹¹⁾. The blockade of Ca^{2+} influx and the inhibition of Ca^{2+} elevation effectively suppressed aggregation and dense-granule release. We found that P-selectin expression was also inhibited with the same treatment. Ami, as a specific inhibitor of Na^+/H^+ exchange, similarly inhibited P-selectin expression. This may result from its effect on the activation of phospholipase A_2 , or the regulation of phosphoinositide cycle in platelets^(12,13). The involvement of protein tyrosine kinase (PTK) in P-selectin expression was further suggested in our experiments by the observation with Gen, a PTK inhibitor, also suppressed P-selectin expression.

In conclusion, multiple signal transduction pathways, including protein tyrosine phosphorylation, Na^+/H^+ exchange, and Ca^{2+} mobilization, mediated PAF-induced P-selectin expression.

REFERENCES

- 1 Zimmerman GA, McIntyre TM, Prescott SM. Adhesion and signaling in vascular cell-cell interactions. *J Clin Invest* 1996; 98: 1699-702.
- 2 Lorant DE, Topham MK, Whatley RE, McEver RP.

- McIntyre TM, Prescott SN, *et al.* Inflammatory roles of P-selectin. *J Clin Invest* 1993; 92: 559-70.
- 3 Weyrich AS, Elstad MR, McEver RP, Zimmerman GA, Moore KL, Morrissey JH, *et al.* Activated platelets signal chemokine synthesis by human monocytes. *J Clin Invest* 1996; 97: 1525-34.
- 4 Hamburger SA, McEver RP. GMP-140 mediates adhesion of stimulated platelets to neutrophils. *Blood* 1990; 75: 550-4.
- 5 Ben-Baruch A, Michiel DF, Oppenheim JJ. Signals and receptors involved in recruitment of inflammatory cells. *J Biol Chem* 1995; 270: 11703-6.
- 6 Rinder CS, Student LA, Bonan JL, Rinder HM, Smith BR. Aspirin does not inhibit adenosine diphosphate-induced platelet α -granule release. *Blood* 1993; 82: 505-12.
- 7 Yi FX, Guo ZG. Histamine is an intracellular messenger mediating PAF-induced rabbit platelet activation. *Chin J Physiol Sci* 1997; 13: 104-10.
- 8 Abrams CS, Ellison N, Budzynski AZ, Shattil SJ. Direct detection of activated platelets and platelet-derived microparticles in humans. *Blood* 1990; 75: 128-38.
- 9 Yi FX, Guo ZG. Platelet-released ADP stabilizes PAF-induced rabbit platelet aggregation by stabilizing intracellular calcium. *Acta Pharmacol Sin* 1998; 19: 379-82.
- 10 Zeng S, Guo ZG. Effects of platelet agonists on intracellular pH in platelets. *Chin J Physiol Sci* 1997; 13: 81-6.
- 11 Brune B, von Appen FV, Ullrich V. Receptor occupancy regulates Ca^{2+} entry and intracellular Ca^{2+} redistribution in activated human platelets. *Biochem J* 1994; 304 (Pt 3): 993-9.
- 12 Sweatt JD, Connolly TM, Cargoe EJ, Limbird LE. Evidence that Na^+/H^+ exchange regulates receptor-mediated phospholipase A_2 activation in human platelets. *J Biol Chem* 1986; 261: 8667-73.
- 13 Luzzatto G, Kroll MH, Zavoico GB, Schafer AI.

Regulation of the phosphoinositide cycle by Na^+/H^+ exchange and intracellular pH in human platelets. *Biochim Biophys Acta* 1991; 1084: 78-86.

R8-950

16

血小板活化因子诱导血小板 P-选择素表达 及相关信号传导机制¹

R973

曾珊, 易富贤¹, 郭兆贵² (湖南医科大学
分子药理研究室, 长沙 410078, 中国)

关键词 血小板激活因子; P-选择素; 阿米洛利;
4,5,7-三羟异黄酮; 依他酸; 流式细胞术; 血小板

药理

目的: 研究 PAF 诱导 P-选择素表达的细胞内信号机制。 **方法:** 置备人血小板悬液, 用流式细胞术测定 PAF 诱导的 P-选择素表达。 **结果:** 用依他酸 $2 \text{ mmol} \cdot \text{L}^{-1}$ 和 BAPTA $200 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ 分别阻断外钙内流和络合内钙, 显著抑制 PAF 诱导的 P-选择素表达 [$(13.3 \pm 0.9) \%$; $(16.8 \pm 1.9) \%$ vs PAF 对照组 $(47.5 \pm 1.3) \%$, $P < 0.01$]。 用阿米洛利 $400 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ 抑制 Na^+/H^+ 交换, Genistein $300 \text{ } \mu\text{mol} \cdot \text{L}^{-1}$ 抑制蛋白酪氨酸磷酸化也均可抑制 PAF 引起的 P-选择素上调 [$(37.5 \pm 2.1) \%$; $(29 \pm 4) \%$ vs PAF 对照组 $(47.5 \pm 1.3) \%$, $P < 0.01$]。 **结论:** 蛋白酪氨酸磷酸化, 细胞内钙动员及 Na^+/H^+ 交换激活中介 PAF 诱导的血小板 P-选择素表达。

(责任编辑 朱倩蓉)