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Dauricine inhibits redistribution of platelet membrane glycoprotein Ⅳ and release of intracellular α-granule thrombospondin induced by thrombin

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KEY WORDS dauricine; blood platelets; glycoproteins; CD36 antigens; thrombin

ABSTRACT

AIM: To study the possibility of dauricine (Dau) inhibiting redistribution of platelet membrane glycoprotein Ⅳ (GPⅣ) and release of intracellular α-granule thrombospondin (TSP) on platelet activation. **METHODS**: Using the flow cytometric assay of washed platelet to record expression of GPⅣ and release of TSP induced by thrombin. **RESULTS**: Dau did not affect GPⅣ and TSP on resting platelet membrane but inhibited redistribution of GPⅣ to the platelet surface and TSP release on activated platelet. There was a marked positive correlation between changes of GPⅣ and TSP ($r = 0.511$, $P < 0.01$). The inhibitory effect of Dau appeared not to be Ca^{2+} concentration-dependent. **CONCLUSION**: Dau inhibited redistribution of GPⅣ and release of intracellular α-granule thrombospondin induced by thrombin.

INTRODUCTION

Platelet membrane glycoprotein Ⅳ (GPⅣ), also known as GPⅢb and CD36, had been proposed as the membrane receptor for thrombospondin (TSP). TSP released from platelet α-granules in platelet activation had been shown to play a role in its binding to GPⅣ on platelet membrane and to be critical for the consolidation of platelet aggregation in thrombosis and hemostasis[1,2]. Dau inhibiting platelet aggregation had been demonstrated in various experiments[3,4]. In the present study, the effect of Dau on redistribution of platelet membrane GPⅣ and release of TSP from intracellular α-granule which bound to platelet surface were studied.

MATERIALS AND METHODS

Reagents and drugs Human α-thrombin, anti-GPⅣ, and anti-TSP monoclonal antibodies (McAb), rabbit anti-mouse IgG antibody labeled with fluorescence isothiocyanate (FITC) were purchased from Sigma (USA). Dauricine (Dau, supplied by Institute of Clinical Pharmacology, Tongji Medical University) was a white powder, M_r 624, mp 103 – 104 ℃, purity > 99 %. It was dissolved in distilled water.

Blood sample and platelet isolation Peripheral blood 9 mL from healthy aspirin-free volunteers (8 males, 8 females, age range from 20 – 60 a) was drawn into 1 mL of acid-citrate-dextrose (trisodium citrate 85, citric acid 71, dextrose 111 mmol·L^{-1}), pH 4.5, and spun (150 × g at 22 ℃ for 15 min) and the platelet-rich plasma (PRP) was removed. The platelets were washed 3 times by centrifugation (2 000 × g at 22 ℃ for 10 min) and resuspension in modified Tyrode's buffer (NaCl 138, KCl 2.9, $NaHCO_3$ 12, NaH_2PO_4 0.4 mmol·L^{-1}, 0.1 % glucose, 0.35 % bovine serum albumin), pH 6.5, with alprostadil (Alp) 50 mg·L^{-1}. The platelet resuspension was at a concentration of 15 × 10^7 cells·L^{-1} in modified Tyrode's buffer, pH 7.3, with $CaCl_2$ 1.5 mmol·L^{-1} or phosphate buffer solution (PBS).

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Activated platelet preparation The platelets were incubated with various concentrations of human α-thrombin or control buffer (37 ℃, 10 min), fixed with 1 % formaldehyde, and washed 3 times. Before the preparation of activated platelet, the platelets were incubated with Dau 50 $\mu mol \cdot L^{-1}$.

Flow cytometric analysis The platelets were incubated with saturating concentration of McAb (anti-GPⅣ McAb at 8 $ng \cdot L^{-1}$ or anti-TSP McAb at 3 $ng \cdot L^{-1}$) at 22 ℃ for 30 min. Background fluorescence, determined by incubation of the platelets with the appropriate normal mouse IgG or conjugate, was subtracted from all samples. Samples were analyzed in an FACSort (Becton Dickinson FACS Systems). For each sample, the fluorescence signal from 10×10^3 cells was measured. Any nonplatelet contaminating cells (including monocytes) were excluded from the analysis as previously described[5].

Statistical analysis Results were expressed as $\bar{x} \pm s$ and compared by variance analysis of rank test. An IBM computer with SAS software package was used for the analysis.

RESULTS

Effect of Dau on distribution of resting platelet GPⅣ and TSP After resting platelet was incubated with Dau, GPⅣ and TSP expressed on platelet membrane did not show difference between resting platelet incubated with Dau and controls (Tab 1).

Tab 1. Effects of Dau on GPⅣ and TSP of the resting platelet. $n = 8$ persons. $\bar{x} \pm s$. $^aP > 0.05$ *vs* control.

	GPⅣ/%	TSP/%
Normal	29.2 ± 5.5	10.4 ± 2.8
Dauricine	28.5 ± 5.4[a]	11.7 ± 2.2[a]

Effect of Dau on redistribution of GPⅣ and release of intracellular α-granule TSP on activated platelet membrane The redistribution of GPⅣ on platelet membrane and the release of intracellular α-granule TSP induced by thrombin 50, 100, 500, 1000, 1500 $U \cdot L^{-1}$ were decreased when platelets were incubated with Dau (Fig 1).

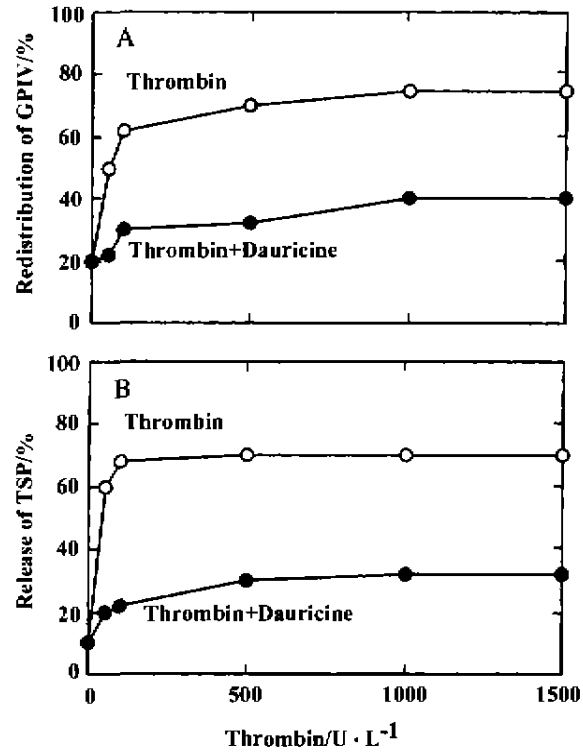

Fig 1. Effect of Dau 50 $\mu mol \cdot L^{-1}$ on redistribution of GPⅣ (A) and release of TSP (B) induced by thrombin.

There was a marked positive correlation between the redistribution of GPⅣ and the release of intracellular α-granule TSP on platelet membrane and the correlation coefficient was 0.511 ($P < 0.01$).

Effect of Dau on GPⅣ and TSP in presence of Ca^{2+} When washed platelets were activated by thrombin in the presence of Ca^{2+} (1.0, 1.5, 2.0 $mmol \cdot L^{-1}$) or PBS, Dau (50 $\mu mol \cdot L^{-1}$) did not inhibit the thrombin-induced upregulation of GPⅣ and TSP in presence of Ca^{2+} (Tab 2).

Tab 2. Effects of Dau 50 $\mu mol \cdot L^{-1}$ on GPⅣ and TSP in presence of Ca^{2+}. $n = 8$ persons. $\bar{x} \pm s$. $^aP > 0.05$ *vs* PBS.

Group	Ca^{2+}/$mmol \cdot L^{-1}$ 1.0	1.5	2.0	PBS
GPⅣ	78.2 ± 8.9[a]	80.1 ± 7.6[a]	79.4 ± 0.1[a]	82.4 ± 9.9
TSP	77.1 ± 7.4[a]	79.8 ± 9.4[a]	78.3 ± 9.4[a]	80.2 ± 8.8

DISCUSSION

Thrombin induces an approximately two-fold increase of GPⅣ and TSP in platelet surface. GPⅣ has been reported to be a TSP receptor and therefore play a role in TSP-dependent platelet aggregation[6]. Many studies have confirmed that GPⅣ and TSP are present in platelet α-granules, as well as in membrane of the open canalicular system, and that thrombin stimulation results in the redistribution of GPⅣ and the release of TSP to platelet plasma membrane[7]. TSP from intracellular α-granule binding to GPⅣ on activated platelet membrane is known to be responsible for the formation of the multiprotein complex (TSP-GPⅣ-GP Ⅱb/Ⅲa-fibrinogen), which is involved in the consolidation of platelet aggregation[8,9]. There are GPⅣ and TSP in intracellular α-granules and the surface-connected canalicular system. Thrombin stimulation results in the redistribution of GPⅣ to the platelet plasma membrane and the release of TSP from α-granules. In fact, it has been speculated that inhibited response of platelet GPⅣ and TSP to thrombin might have a protective effect against atherosclerosis.

Dau was isolated from rhizome of *Memispermum dauricum* DC and its effect on inhibiting platelet aggregation had been demonstrated in various animals[3,4]. The mechanism of Dau inhibiting platelet aggregation was associated with decreased platelet thromboxane B_2 production and cytoplasmic Ca^{2+} level. These changes occurred in the primary platelet activation. In this experiment, Dau markedly inhibited the redistribution of GPⅣ and the release of intracellular α-granule TSP on platelet membrane, which appeared to be Ca^{2+} concentration-independent. These changes are different from the primary process of activated platelet. It is suggested that Dau as an anti-platelet aggregation drug should contribute to blocking the consolidation of platelet aggregation in thrombosis diseases.

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蝙蝠葛碱抑制凝血酶诱导的血小板膜糖蛋白Ⅳ再分布和胞内 α-颗粒凝血酶敏感蛋白释放

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关键词 蝙蝠葛碱；血小板；糖蛋白类；CD36 抗原；凝血酶

目的：探讨蝙蝠葛碱（Dau）对血小板聚集功能的影响．观察 Dau 对血小板膜糖蛋白Ⅳ（GPⅣ）再分布和胞内 α-颗粒凝血酶敏感蛋白（TSP）释放的抑制作用．**方法**：应用流式细胞仪分别检测静息血小板GPⅣ分布、TSP 释放，活化血小板膜GPⅣ再分布和 α-颗粒 TSP 释放．**结果**：Dau 并不影响静息血小板膜GPⅣ和 TSP 分布，而对活化血小板膜GPⅣ再分布和细胞内 α-颗粒 TSP 释放具有明显的抑制作用，且两者的抑制作用呈显著正相关（$r = 0.511$，$P < 0.01$），这种抑制作用并不受 Ca^{2+} 浓度的影响．**结论**：Dau 抑制活化血小板膜GPⅣ再分布和胞内 α-颗粒 TSP 释放．

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