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Platelet Integrin α IIb β 3 Inhibitor Rescues Progression of Apoptosis in Human Platelets

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Data Interpretation D
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Background: Apoptosis plays an important role in the physiology of platelet function. We aimed to detect the effect of the platelet integrin α IIb β 3 inhibitor, tirofiban, on apoptotic events, including mitochondrial inner-membrane potential ($\Delta\Psi_m$), phosphatidylserine (PS) exposure on platelet surface, and the generation of reactive oxygen species (ROS), when washed platelets were stimulated with thrombin.

Material/Methods: The study included washed platelets from healthy humans, divided into 4 groups: vehicle, and tirofiban (0.05 μ g/ml, 0.25 μ g/ml, and 0.5 μ g/ml). Platelets were pretreated with vehicle or tirofiban and incubated at 37°C with agitation for 6 h and 24 h. Before thrombin addition, the vehicle group divided into 2 equal groups. Except one vehicle group, the other 4 groups were all stimulated with thrombin (1 U/ml) for 30 min at 37°C. Using flow cytometry, we studied the $\Delta\Psi_m$ and PS exposure on platelet surfaces, and the generation of ROS in platelets.





Results: We observed that at the time of 6 h and 24 h, thrombin-stimulated vehicle platelets induced significant depolarization of $\Delta\Psi_m$, higher PS exposure, and increased ROS production compared with the vehicle group ($P < 0.01$). However, the tirofiban group had significantly more recovery of $\Delta\Psi_m$, PS exposure, and ROS production compared with the thrombin group ($P < 0.01$).

Conclusions: The platelet integrin α IIb β 3 inhibitor, tirofiban, inhibits the depolarization of $\Delta\Psi_m$, PS exposure on platelet surface, and ROS production when stimulated with thrombin. These results suggest that α IIb β 3 inhibitor inhibits the initiation of apoptosis in platelets, showing a potential clinical application of tirofiban as an apoptosis inhibitor.

MeSH Keywords: **Apoptosis • Flow Cytometry • Mitochondrial Membranes • Phosphatidylserines • Platelet Aggregation Inhibitors**

Abbreviations: $\Delta\Psi_m$ – mitochondrial inner-membrane potential; **PS** – phosphatidylserine; **TF** – Tirofiban; **ROS** – reactive oxygen species; **MTB** – modified tyrode's buffer

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Background

Platelets play an important role in physiological hemostasis and thrombosis. A recent study confirmed that platelets also contribute to many inflammatory and immune disorders, including diverse cardiovascular diseases such as myocardial infarction and stroke [1–3]. Antiplatelet therapy plays a key role in prevention of thrombotic events in conjunction with many other antiplatelet drugs. These in turn develop a strong specificity and show fewer adverse effects; therefore, these drugs have become a popular research topic. Platelet integrin, α IIb β 3, has received increasing attention, plays an important role in platelet aggregation, and prevent generation of outside-in signaling to induce platelet apoptosis [2]. Integrin α IIb β 3 antagonist was developed decades ago and was in common clinical use, along with target-identical receptors, eptifibatide and tirofiban. Tirofiban is able to block α IIb β 3 binding to fibrinogen, and thereby effectively prevents platelet aggregation [1,4]. Interestingly, besides the effect on blocking aggregation, Leytin et al. reported that tirofiban was capable of inhibiting apoptosis-inactivating caspase-3 activity when human platelets were stimulated with thrombin or calcium ionophore A23187 [5]. Consistent with the inhibitory effect on platelet apoptosis incurred by agonists, it has been reported that tirofiban counteracts endothelial cell apoptosis [6].

Two main pathways evoke the process of apoptosis in the clearance of eliminated platelets. The first is the extrinsic pathway, which occurs by ligands that connect with the death receptors on the platelet surface, and which belong to the tumor-necrosis factor (TNF) superfamily. This results in activating a death signal transfer to phagocytes, leading to phagocytosis of the activated platelets. The second is the intrinsic pathway, which is dependent on mitochondrial function disruption [7,8]. The intrinsic pathway initiated by the activated platelets releases cellular signal transfer to the mitochondria. This triggers the depolarization of mitochondrial inner-transmembrane potential ($\Delta\Psi_m$), and pro- and anti-apoptotic proteins of Bcl-2 family disorders, which subsequently release other pro-apoptotic proteins, including cytochrome C and activated caspase-9 [9–13]. Due to the depolarization potential of the inner-transmembrane of mitochondria, there occurs a hallmark event in the initiation of platelet apoptosis, which is then characterized as the indicator of early apoptosis [9,14]. Leytin et al. showed that tirofiban reduced the caspase-3 activation induced by antagonists [5], but the effect of tirofiban on the initiation of apoptosis is still unclear. Downstream phosphatidylserine exposure [14,15] is a marker of early apoptosis in platelets as well. Phosphatidylserine is only present on the inner plasma membrane in proper functioning of intact cells, whereas apoptosis incurs aberrant location of phosphatidylserine on the outer plasma membrane leaflet, leading to elimination of adjacent cells. Reactive oxygen species (ROS) are produced and released by stimulated platelets and take part in the development

of apoptosis [16]. Reactive oxygen species, including hydrogen peroxide (H_2O_2), play a crucial role in intra-platelet signaling and inducing activation and apoptosis [16,17].

Thrombin induces apoptosis in platelets [18], and reactive oxygen species participate in the process. Recently, tirofiban has been implicated in the generation of reactive oxygen species in ischemia/reperfusion-induced renal injury [19], but the effect of tirofiban on platelets stimulated with thrombin is not clear. Hence, to explore the effect of tirofiban on the initiation and progression of apoptosis, we studied the alteration of depolarization of mitochondrial inner-transmembrane potential, phosphatidylserine exposure, and reactive oxygen species generation in platelets to detect the potential and the mechanism of tirofiban in early apoptosis in the activated platelets.

Material and Methods

Material

We washed platelets from healthy adult volunteers who did not drink alcohol or take any drugs

Reagents

Anti- β -actin, 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1) was purchased from Beyotime Institute of Biotechnology (Beyotime, Haimen, China). Fluorescein isothiocyanate (FITC)-conjugated Annexin-v antibody was purchased from Jiamay Biotech CO. LTD (Jiamay, Beijing, China). Thrombin was purchased from Sigma (Missouri, St. Louis, MO). Tirofiban hydrochloride and sodium chloride injection was purchased from Grand Pharma (China) Co. LTD (Wuhan, Hubei, China). 2',7'-Dichlorofluorescein diacetate (DCFH-DA) was purchased from Sigma (St. Louis, MO).

Methods

Washed platelets preparation

Fresh venous blood from healthy adult volunteers was anticoagulated with 1/7 volume of acid-citrate dextrose. Platelet-rich plasma (PRP) was obtained from fresh whole blood after being centrifuged at 300 g for 10 min. Isolated platelets were obtained from PRP after centrifuging at 1500 g for 2 min, and then were washed twice with CGS buffer (0.123 M NaCl, 0.033 M D-Glucose, 0.013 M trisodium citrate, pH 6.5) and resuspended into modified Tyrode's buffer (MTB) (2.5 mM Hepes, 150 mM NaCl, 2.5 mM KCl, 12 mM $NaHCO_3$, 5.5 mM D-glucose, 1 mM $CaCl_2$, 1 mM $MgCl_2$, pH 7.4). Platelet concentration was adjusted to 3×10^8 /ml. All materials were sterile in this process. The platelet suspensions were held at room temperature for 1 h.

Treatment of platelets with tirofiban and thrombin

Washed platelets were divided into 4 groups: (i) vehicle (normal saline), (ii) tirofiban 0.05 μ g/ml, (iii) tirofiban 0.25 μ g/ml, and (iv) tirofiban 0.5 μ g/ml. All platelet samples were placed in equal aliquots and stored at 37°C with agitation for 6 h and 24 h. The vehicle group was divided into 2 equal aliquots, with thrombin (1U/ml) placed into one vehicle group and retained the other vehicle group. The stimulated platelets were kept at 37°C for 30 min. Thus, we now had 5 groups: the vehicle group without thrombin (Vehicle), the vehicle group with thrombin, tirofiban 0.05 μ g/ml with thrombin, tirofiban 0.25 μ g/ml with thrombin, and tirofiban 0.50 μ g/ml with thrombin. Platelets undergoing different interventions were placed into equal aliquots to determine mitochondrial inner-transmembrane potential ($\Delta\Psi$ m) depolarization assay, phosphatidylserine exposure assay, and reactive oxygen species generation assay.

Mitochondrial inner-transmembrane potential ($\Delta\Psi$ m) depolarization assay

Platelet samples from 4 groups were placed into equal aliquots at 6 h and 24 h. Platelets were kept at 37°C for 30 min to stimulate thrombin. Depolarization of mitochondrial inner-transmembrane potential in different groups was detected by JC-1, which is a lipophilic cationic probe monomer. JC-1 stock solution (2 mg/ml) was diluted with DMSO to 0.2 mg/ml, and diluted with MTB; the JC-1 work solution was 2 μ g/ml. JC-1 fluorochrome was added into the 5 groups and kept for 10 min in the dark. Measurement of the mitochondrial inner-transmembrane potential was evaluated by flow cytometry. Depolarization of mitochondrial inner-transmembrane potential in platelets is characterized by the conversion of JC-1 dye aggregates to JC-1 monomers, reflecting the reduced red fluorescence (FL2) of JC-1 aggregates from green fluorescence (FL1). Depolarization of mitochondrial inner-transmembrane potential was quantified as increase of the percentage of depolarized cells, which reflects the decrease in content of JC-1 aggregates when the inner-mitochondrial membrane becomes depolarized.

Phosphatidylserine (PS) exposure assay

Platelet samples from 4 groups were taken with same aliquots at 6 h and 24 h. The vehicle group was divided into 2 equal aliquots, then we added thrombin (1 U/ml) into 4 groups (but not the vehicle group). Platelets were kept at 37°C for 30 min to stimulate thrombin. We added fluorescein isothiocyanate (FITC)-conjugated Annexin-V antibody and 150 μ l 1 \times binding into the 5 groups for 20 min, then added 350 μ l 1 \times binding to end the reaction. Phosphatidylserine exposure was detected by flow cytometry and quantified the expression as positive Annexin-V platelets.

Reactive oxygen species (ROS) generation assay

Platelet samples from 4 groups were aliquoted at 6 h as previously described. ROS production in platelets was detected using an ROS assay kit and were analyzed by flow cytometry. Briefly, washed platelet samples were incubated with DCFH-DA (20 μ M) at 37°C for 45 min in the dark. Subsequently, they were centrifuged and resuspended with MTB. The vehicle group was divided into 2 equal aliquots, then we added thrombin (1 U/ml) into the other 4 pretreated groups with or without tirofiban (but not the vehicle group) at 37°C for 30 min, and reactive oxygen species generation was detected by flow cytometry. The reactive oxygen species stimulation index was based on the fluorescent values of the unstimulated vehicle platelet group, set as 1, and the other groups were assessed as fold-change compared with the unstimulated vehicle group. DCFH-DA diffuses across the platelet cytomembrane and is hydrolyzed by non-specific cellular esterases in platelet cytoplasm to form non-fluorescent dichlorofluorescein (DCFH), which loses the ability to diffuse across the cytomembrane. Reactive oxygen species rapidly induces DCFH and undergoes one-electron oxidation to generate DCF, which is a fluorescent compound. Therefore, the reactive oxygen species production is reflected by an increase of DCF radicals in platelets and was detected by flow cytometry.

Statistical analysis

Data are shown as mean \pm standard deviation (SD). The statistical significance of the differences between the 5 groups was measured by one-way ANOVA. A P-value <0.05 was regarded as significant.

Results

Incubation with different concentrations of tirofiban for 6 h inhibits the depolarization of mitochondrial inner-membrane potential ($\Delta\Psi$ m) in thrombin-stimulated human platelets

After incubation with vehicle or tirofiban for 6 h at 37°C, thrombin triggers a loss of mitochondrial inner-transmembrane potential in the vehicle platelets when compared with the vehicle group without thrombin. Interestingly, platelets incubated with tirofiban for 6 h were capable of inhibiting thrombin-induced depolarization of mitochondrial inner-transmembrane potential when compared with thrombin-stimulated platelets without tirofiban (Figure 1A.) (P<0.01). There were no significant differences in the effect of the 3 concentrations of tirofiban on inhibitory effects (Figure 1B).

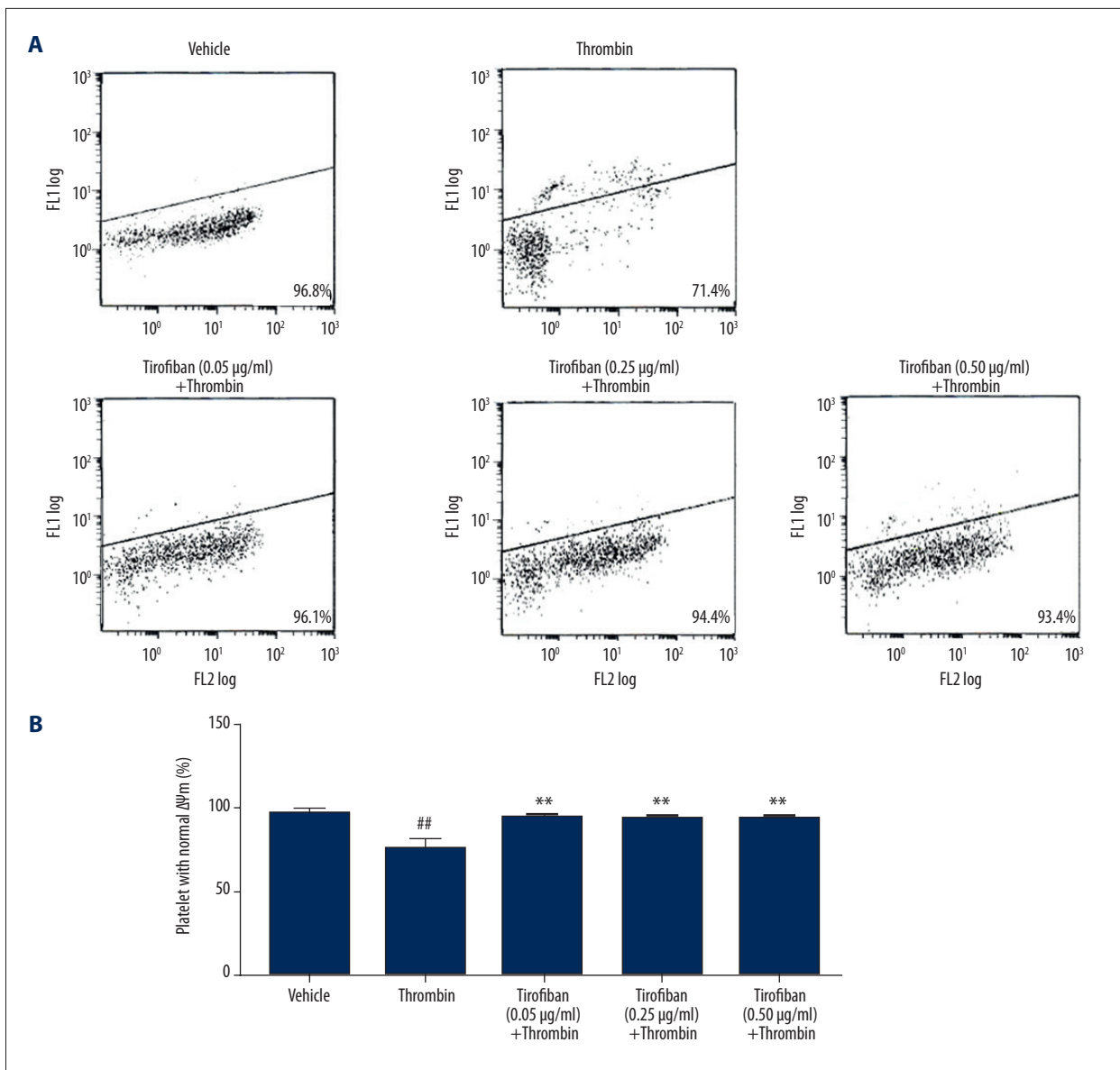


Figure 1. The effect of tirofiban on depolarization of mitochondrial inner-membrane potential ($\Delta\Psi_m$) in thrombin-stimulated human platelets. **(A)** Typical dot plots are based on washed platelets held at 37°C for 6 h with agitation and treated with vehicle (normal saline), thrombin (normal saline), or tirofiban (0.05 μ g/ml, 0.25 μ g/ml, 0.5 μ g/ml respectively) before adding thrombin (1 U/ml)-stimulated platelet, except vehicle group. Platelets were kept at 37°C for 30 min and stimulated with thrombin or not. JC-1 was added to different groups to determine mitochondrial inner-transmembrane potential collapse. **(B)** Quantitative analysis of different tirofiban concentration interventions are shown as mean \pm SD, and P-value. ## Vehicle group compared with thrombin group, $P < 0.01$; ** Tirofiban group compared with thrombin group, $P < 0.01$.

Incubation with different concentrations of tirofiban for 6 h inhibits phosphatidylserine (PS) exposure in thrombin-stimulated human platelets

In our observation, we noticed that thrombin triggered a higher phosphatidylserine exposure in the vehicle group compared to the vehicle group without thrombin, while pre-incubated tirofiban with different concentrations in platelets for 6 h were

capable of inhibiting thrombin-induced phosphatidylserine exposure when compared with the thrombin-stimulated platelets in the vehicle group ($P < 0.01$) (Figure 2A). No significant differences were observed in the effect of the 3 tirofiban concentrations on inhibition (Figure 2B).

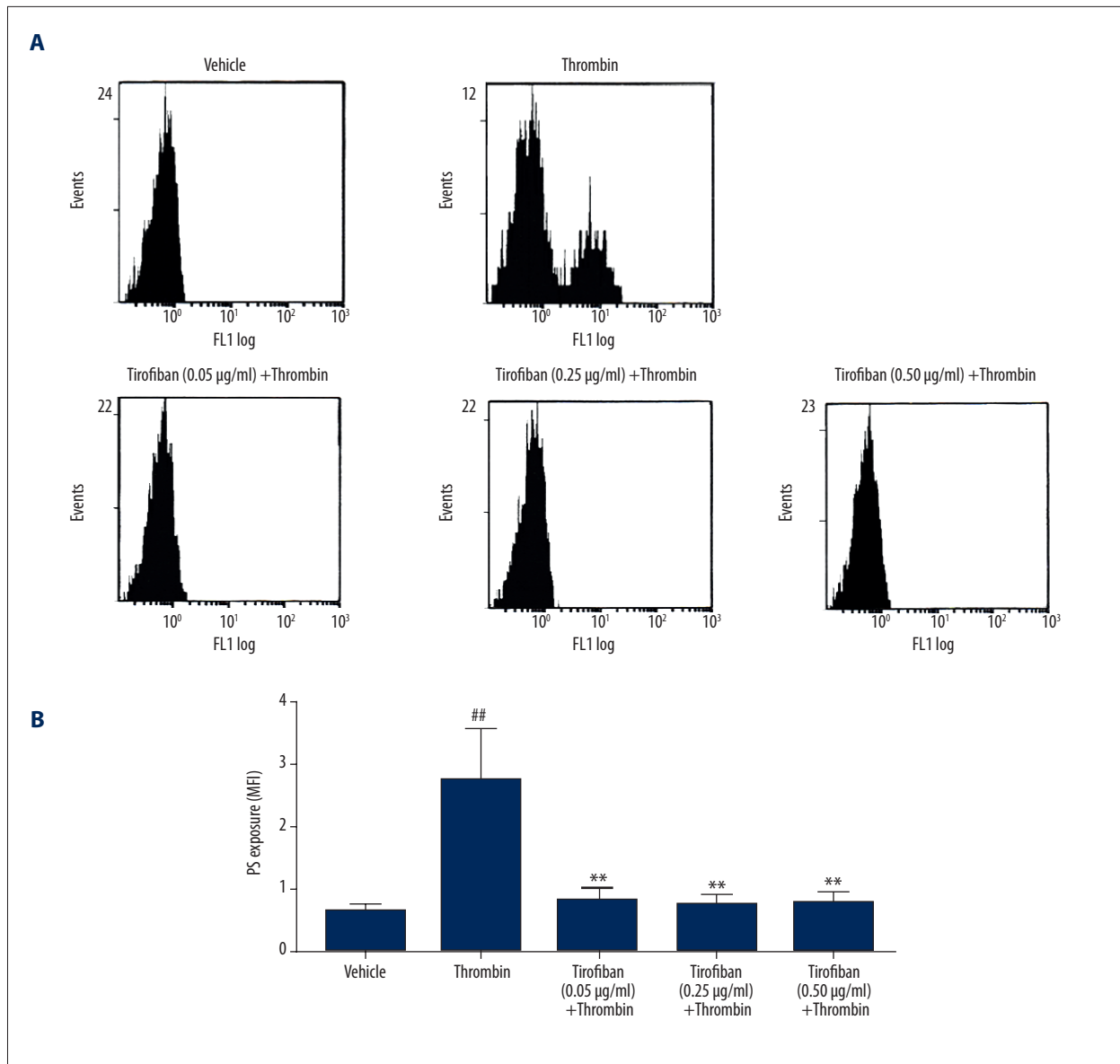


Figure 2. The impact of tirofiban on phosphatidylserine (PS) exposure in thrombin-stimulated human platelets. **(A)** Typical histogram graph shows washed platelets held at 37°C for 6 h with agitation, treated with vehicle (normal saline), thrombin (normal saline), or tirofiban (0.05 µg/ml, 0.25 µg/ml, and 0.5 µg/ml, respectively) before adding thrombin (1 U/ml). We then stimulated the 4 groups of platelets (except vehicle group) with thrombin or not for 30 min at 37°C. FITC-Annexin-V antibody was added to the 5 groups to determined phosphatidylserine exposure. **(B)** Quantitative analysis of different tirofiban concentration interventions are shown as mean \pm SD and P-value. ## Vehicle group compared with thrombin group, $P < 0.01$; ** Tirofiban group compared with thrombin group, $P < 0.01$.

The alteration of mitochondrial inner-membrane potential ($\Delta\Psi_m$) in thrombin-stimulated platelets along with different periods of preincubation with or without tirofiban

We studied platelets pre-incubated with or without tirofiban for 6 h or 24 h. In the vehicle group, thrombin induced significant depolarization of mitochondrial inner-transmembrane

potential compared with the vehicle group without thrombin. However, platelets pre-incubated with tirofiban significantly increased mitochondrial inner-membrane potential (Figure 3A). The inhibitory effect of tirofiban on thrombin induced depolarization of mitochondrial inner-transmembrane potential still existed of up to 24 h (Figure 3B).

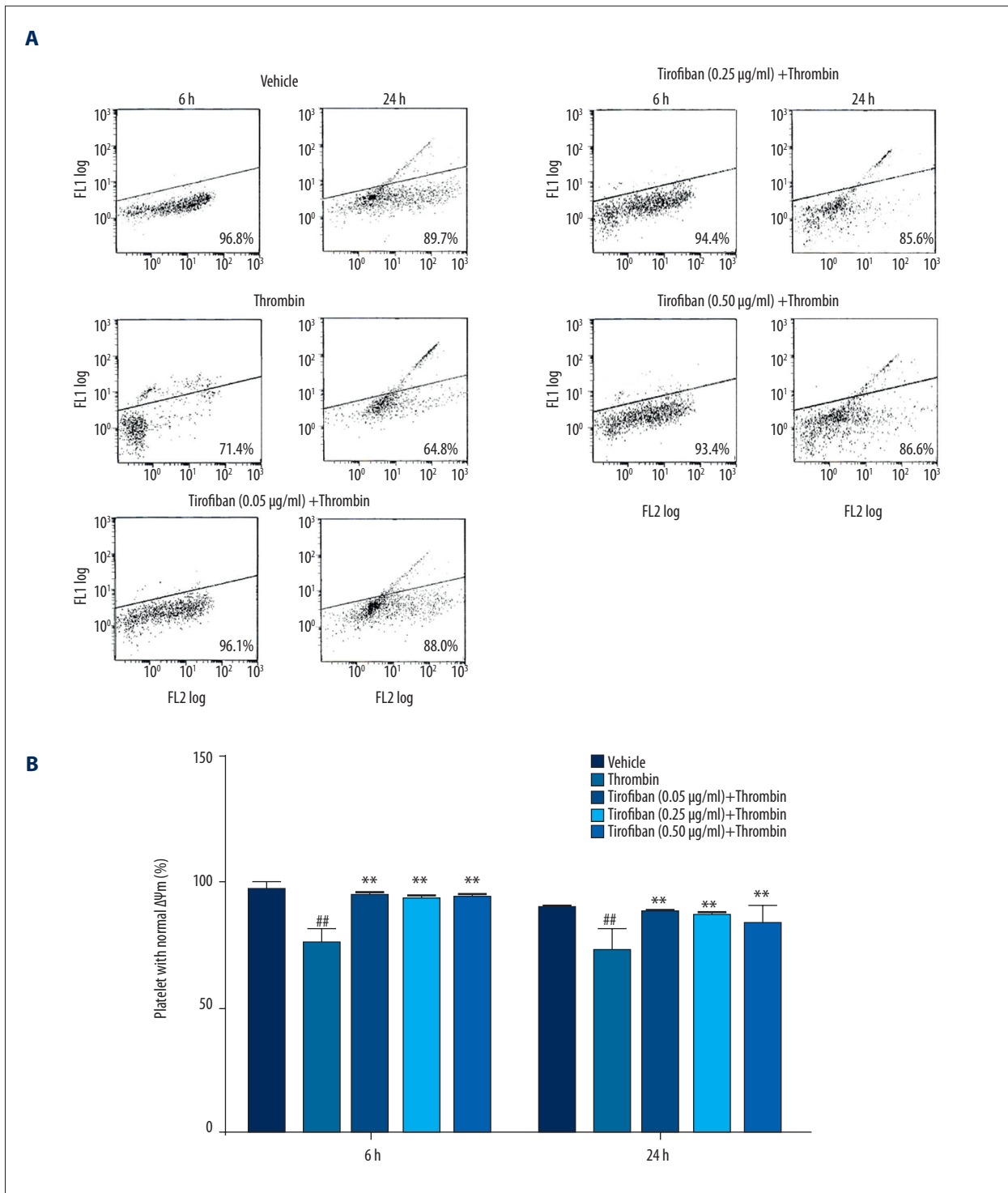


Figure 3. The alteration of mitochondrial inner-membrane potential ($\Delta\Psi_m$) depolarization in thrombin-stimulated platelets undergoing different periods of preincubation with tirofiban. **(A)** Typical dot plots are displayed of washed platelets held at 37°C for 6 h or 24 h with agitation, treated with vehicle (normal saline), thrombin (normal saline), tirofiban (0.05 $\mu\text{g/ml}$, 0.25 $\mu\text{g/ml}$, 0.5 $\mu\text{g/ml}$, respectively) before adding thrombin (1 U/ml). We then stimulated the 4 groups platelets (except vehicle group) with thrombin or not under for 30 min at 37°C. JC-1 was added into platelets to determine mitochondrial inner-transmembrane potential. **(B)** Quantitative analysis of the 5 group at 6 h and 24 h are shown as mean \pm SD and P-value. ## Vehicle group compared with thrombin group, $P < 0.01$; ** Tirofiban group compared with thrombin group, $P < 0.01$.

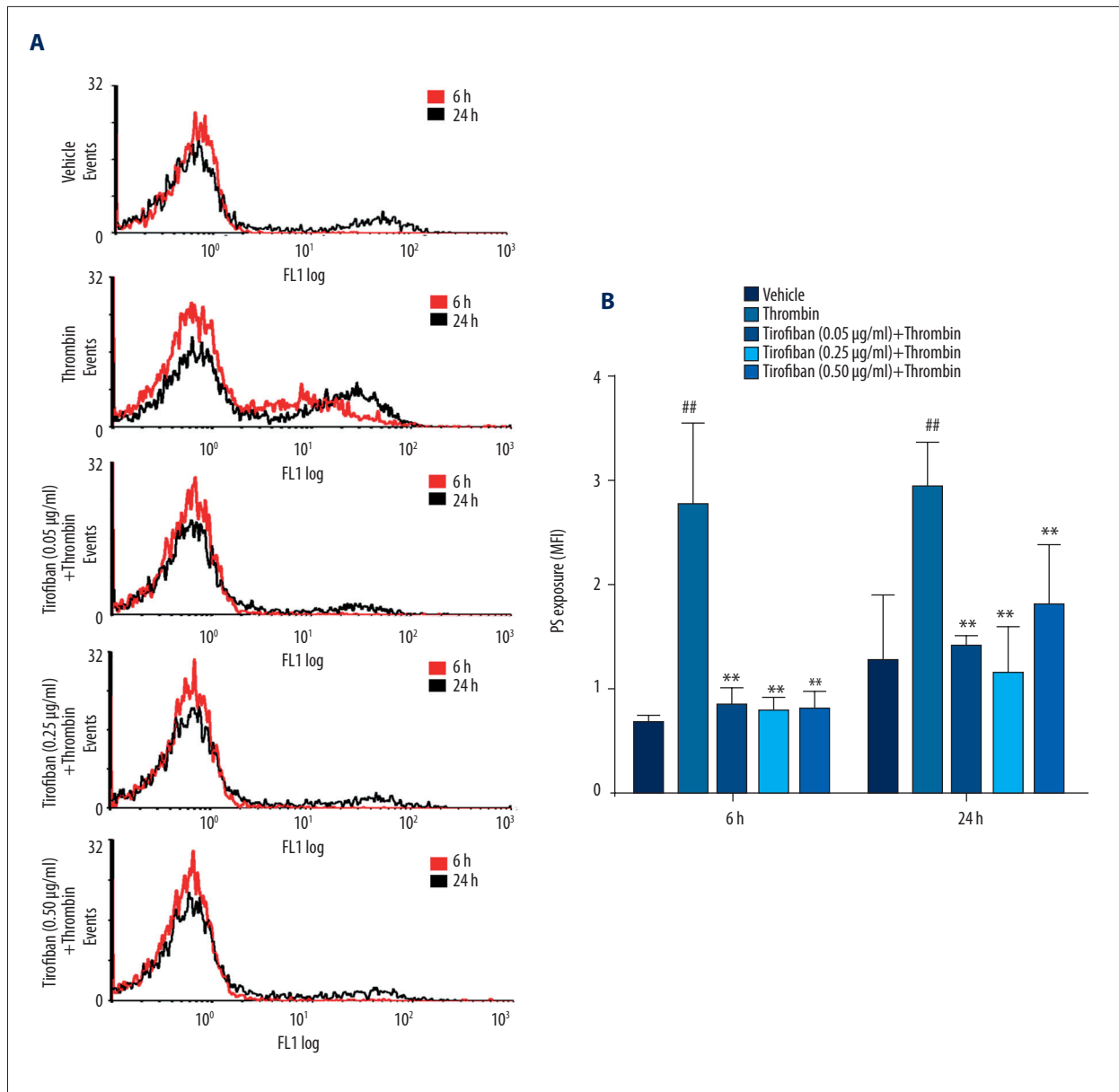


Figure 4. The alteration of phosphatidylserine (PS) in thrombin-stimulated platelets undergoing different periods of preincubation with tirofiban. **(A)** Typical histogram graphs are displayed showing washed platelets held at 37°C for 6 h or 24 h with agitation, treated with vehicle (normal saline), thrombin (normal saline), or tirofiban (0.05 μ g/ml, 0.25 μ g/ml, 0.5 μ g/ml, respectively) before adding thrombin (1 U/ml). We then stimulated the 4 groups of platelets (except vehicle group) with thrombin or not, for 30 min at 37°C. FITC-Annexin-V antibody was added into platelets to determine phosphatidylserine exposure. **(B)** Quantitative analysis of the 5 group at 6 h and 24 h are shown as mean \pm SD and P-value. ^{##} Vehicle group compared with thrombin group, $P < 0.01$; ^{**} Tirofiban group compared with thrombin group, $P < 0.01$.

The alteration of phosphatidylserine (PS) exposure in thrombin-stimulated platelets along with different periods of preincubation with or without tirofiban

Similarly, with detection of depolarization of mitochondrial inner-transmembrane potential, we carefully explored the effect of tirofiban on phosphatidylserine exposure for different

preincubation periods. Our results are consistent with mitochondrial inner-transmembrane potential outcomes. We also studied platelets pre-incubated with or without tirofiban for 6 h or 24 h. In the vehicle group, thrombin induced significantly higher phosphatidylserine exposure compared with the vehicle group without thrombin. However, platelets pre-incubated with tirofiban significantly reduced phosphatidylserine

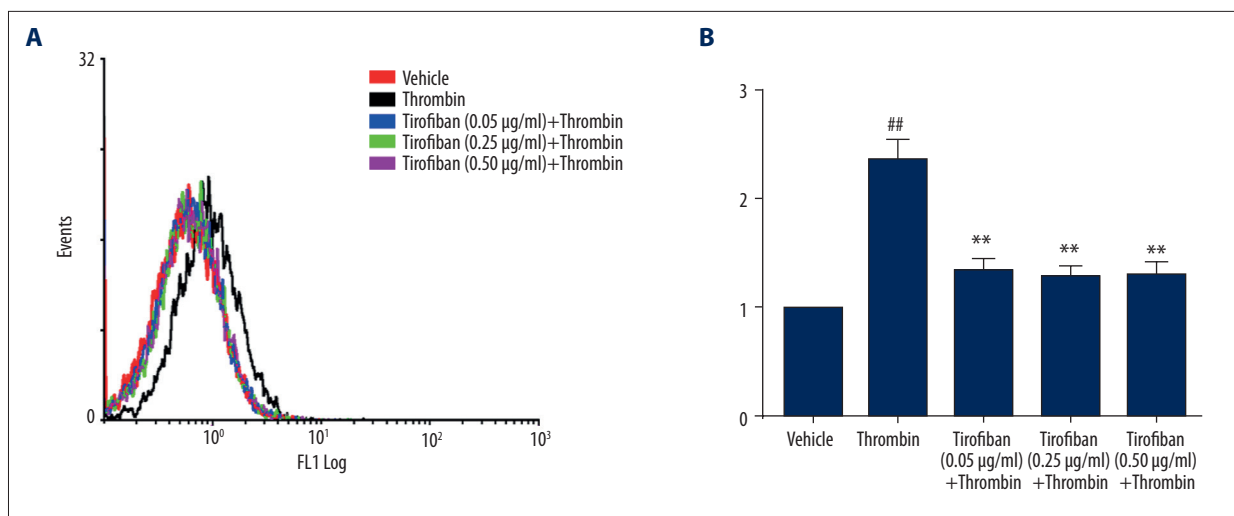


Figure 5. (A, B) The effect of tirofiban on the generation of reactive oxygen species in thrombin-stimulated human platelets. The histogram shows washed platelets held at 37°C for 6 h with agitation and treated with vehicle (normal saline), thrombin (normal saline), or tirofiban (0.05 µg/ml, 0.25 µg/ml, 0.5 µg/ml, respectively) before adding thrombin (1 U/ml) (except vehicle group). Platelets were pre-incubated with 20 µM DCFH-DA before stimulation. We determined the reactive oxygen species generation by flow cytometry. Data are shown as mean \pm SD and P-value. ## Vehicle group compared with thrombin group, $P < 0.01$; ** Tirofiban group compared with thrombin group, $P < 0.01$.

exposure on the platelet surface (Figure 4A). The inhibitory effect of tirofiban on thrombin induced phosphatidylserine exposure still existed up to 24 h (Figure 4B).

The generation of reactive oxygen species in thrombin-stimulated platelets along with preincubation

We discovered that thrombin triggered higher reactive oxygen species production in the thrombin group compared to the vehicle group without thrombin (Figure 5A), while pre-incubated tirofiban with different concentrations in platelets for 6 h were capable of inhibiting thrombin-induced reactive oxygen species production when compared with the thrombin-stimulated platelets in the vehicle group ($P < 0.01$) (Figure 5B). These results suggest that tirofiban maybe has an effect on the generation of reactive oxygen species induced by thrombin in platelets.

Discussion

Platelets play a critical role in the progression of atherosclerotic thrombosis [1,2], and antiplatelet drugs are becoming more and more important in preventing the formation of thrombosis.

To evaluate the function and quality of platelets, the extent of apoptosis is an important index to which researchers should pay attention. In apoptosis, also referred to as programmed cell death, stimulation is generally classified as being either physiological or weak. In the absence of nuclear platelets, we characterized the apoptosis markers, except the nucleus, which

includes phosphatidylserine exposure on platelet surface and the depolarization of mitochondrial inner-membrane potential. In our experiment, we made use of thrombin to monitor the pathological conditions to induce platelet apoptosis [18,20,21]. Apoptotic parameters were preincubation conditions for 6 h or 24 h, followed by 1 U/ml thrombin stimulation for 30 min at 37°C. In comparison to thrombin-stimulated platelet preincubation without tirofiban, tirofiban preincubation with platelets significantly decreased thrombin-induced apoptosis markers, including the depolarization of mitochondrial inner-transmembrane potential and phosphatidylserine exposure inhibition. Washed platelets were titrated with different tirofiban concentrations (0.05 µg/ml, 0.25 µg/ml, and 0.5 µg/ml) and incubated in the experimental setting. We found that the low concentration of tirofiban was able to control platelet apoptosis. These data suggest that thrombin induced platelet apoptosis, including marked depolarization of mitochondrial inner-transmembrane potential, phosphatidylserine exposure and reactive oxygen species production. Platelet preincubation with tirofiban effectively recovers the mitochondrial inner-transmembrane potential, inhibits phosphatidylserine exposure and reactive oxygen species production compared with thrombin-stimulated platelets without tirofiban administration. Along with the different durations of preincubation with tirofiban, we observed that tirofiban preincubation for 6 h or 24 h at 37°C both recover impaired platelet function rescue from apoptosis induced by thrombin. We pre-incubated platelets with tirofiban for 6 and 24 h to investigate the long-term efficacy of tirofiban on platelet apoptosis, and demonstrated that the inhibitory effect of tirofiban on platelet apoptosis still existed up to

24 h. Interestingly, we applied different effective concentrations of tirofiban to detect the effective dosage in protecting the platelet from apoptosis [13,22]. Increased storage time of platelets induces more apoptosis [23–26]. We also tested the effect of tirofiban on storage-induced platelet apoptosis and found that none of the tirofiban doses used in this study affected platelet apoptosis caused by long-term incubation (data not shown), suggesting that tirofiban only inhibits thrombin-induced platelet apoptosis.

These observations suggest that besides inhibiting caspase-3 activation, tirofiban is also capable of decreasing depolarization of mitochondrial inner-transmembrane potential, suppressing phosphatidylserine extravasation and reactive oxygen species production. Furthermore, tirofiban not only inhibits the effector phase of apoptosis, but also suppresses the initiation of apoptosis.

These results suggest that tirofiban inhibits platelet apoptosis via the intrinsic mitochondria-dependent pathway. Depolarization of mitochondrial inner-transmembrane potential is a hallmark manifestation of the initiation of apoptosis, which can be used to precisely and rapidly monitor mitochondrial function [7,8,14,27]. In addition, after mitochondrial function disorders, B-cell lymphoma-2 (BCL-2) family proteins, including anti-apoptotic and pro-apoptotic proteins, exist in mitochondrial membranes that participate in mediating platelet apoptotic progression. The anti-apoptotic proteins, including BCL-2 and Bcl-X_L, are able to bind to the corresponding pro-apoptotic events and reduce the activation of events to recover stable condition. It is crucial to affect platelet survival. Pro-apoptotic proteins, including BAK, BAX, and BAD, are overexpressed to facilitate apoptosis via launching mitochondrial membrane permeabilization formation to disrupt mitochondrial integrity and activate other apoptotic factors [13,19]. The contents of these proteins need to be further determined, and may further verify our suspicion. The other apoptotic marker, phosphatidylserine exposure on platelets, reflects apoptotic alteration in the plasma membrane [15]. Phosphatidylserine exposure exchanges from inner to outer plasma membrane can be recognized by phagocytes to eliminate platelets [27,28]. Consistent with mitochondrial inner-transmembrane potential, we observed that tirofiban was affected the inhibition of phosphatidylserine exposure, suggesting that tirofiban not only protects the initiation of platelets, but also protects the downstream reaction, including inhibition of the crucial factor, phosphatidylserine exposure [12,14]. Interesting, it has been proved that thrombin caused apoptosis via generating reactive oxygen species [18]. It has been reported that tirofiban inhibits reactive oxygen species production in ischemia/reperfusion-induced renal injury [19]. We found that tirofiban inhibits the reactive oxygen species generation induced by thrombin (Figure 5), which suggests that tirofiban affects the process of apoptosis through regulating reactive oxygen species production.

The suppression effect of tirofiban on thrombin occurs through platelet depolarization of mitochondrial inner-transmembrane potential, and phosphatidylserine exposure suggests that tirofiban may affect thrombin stimulating platelet apoptosis, especially in the initiation of platelet apoptosis. Transmembrane potential and phosphatidylserine exposure is caused by apoptotic signal transduction through platelet surface receptors. In addition, GPIIb/IIIa integrin signaling is subsequently launched to activate platelets; therefore, the inside-out signaling through protein kinases release activated platelet surface receptors to bind to ligands. Furthermore, these ligands bind to receptors by activated outside-in signaling, thus accelerating platelets apoptosis [16,29–31]. Reports have suggested that phosphatidylserine exposure has a connection with GPIIb/IIIa when platelets are stimulated with agonists [32]. When we used GPIIb/IIIa inhibitor incubated with platelets, we found that tirofiban blocks ligands, including fibrin and vWF, binding to GPIIb/IIIa receptors, which interdict the outside-in signaling to alleviate platelet apoptosis.

Apoptosis in platelets plays a key role in the storage of blood, progression of atherosclerosis, and idiopathic thrombocytopenic purpura (ITP) [8,11]. We found that the platelet integrin α IIb β 3 antagonist, tirofiban, had an inhibitory effect on apoptosis of platelets, which may produce new insight into clinical use of the drug. Numerous studies had shown that phosphatidylserine exposure gradually increased along with storage time. Whether the integrin α IIb β 3 antagonist has an effect on inhibiting apoptosis in platelets requires further exploration.

Apoptosis has a close connection with the initiation and progression of atherosclerosis [11]. The impaired endothelial cells or pathological high shear of circulation induces platelet GPIIb/IIIa to combine with von Willebrand Factor (vWF), causing platelets to adhere to the damaged vascular surface [18]. Platelets initiate the signal transduction pathway to activate more platelets, thus activating integrin α IIb β 3, which combines with fibrinogen and vWF to form stable thrombosis. At the start of the disease, these factors (thrombin, collagen, and high shear) activate the intrinsic mitochondria-dependent pathway to induce apoptosis in platelets [8,29].

GPIIb/IIIa inhibitor is able to inhibit the intrinsic mitochondria-dependent pathway and inhibit reactive oxygen species production to suppress apoptosis in platelets. Our result helps explain the recovery from apoptosis in atherosclerosis.

Conclusions

Our data suggest that tirofiban has a direct effect on thrombin-inducing platelet apoptosis parameters, including depolarization of inner-mitochondrial transmembrane potential

and phosphatidylserine extravasation, which are involved in the initiation and process of apoptosis, and affects reactive oxygen species generation. It is also important to understand the mechanism and the regulation of factors in platelet-induced apoptosis, which also gives new insight to explore a novel treatment for apoptosis. The outcomes may be applied with related diseases and in the development of novel drugs,

and suggest that tirofiban may be considered as a modulator of apoptosis in platelets.

Conflict of interests

The authors have no conflicts of interest to declare.

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