Lyophilized human platelets support thrombosis unlike normal platelets in the presence of GPIIb/IIIa antagonists

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INTRODUCTION

GPIIb/IIIa inhibitors block platelet fibrinogen and vWF receptors thereby reducing thrombosis. In an emergency, these agents must be overcome to stop bleeding.

AIMS

The aim of this study was to determine if Thrombosomes®, a lyophilized human platelet (LHP) hemostatic agent under clinical development, were resistant to the effect of GPIIb/IIIa antagonists and therefore restore hemostasis associated with GPIIb/IIIa antagonist mediated bleeding.

METHODS

GPIIb/IIIa inhibitors representative of different classes of therapeutic molecules, an antibody (AP-2), a cyclic heptapeptide (eptifibatide) and a non-peptide small molecule (tirofiban) were tested for their ability to inhibit aggregation of donor and platelet rich plasma (PRP). Doses of each drug shown to inhibit aggregation of platelets were used to treat PRP and inhibit thrombus formation on the T-TAS® flow system over thromboplastin-collagen coated microcapillaries. Single donor apheresis platelets (standard of care for drug reversal) and LHP were compared for their ability to recover loss of occlusion in the presence of each drug.

RESULTS

Fresh drawn platelet rich plasma aggregate in response to collagen (figure 1a) which can be completely inhibited by tirofiban, eptifibatide or AP-2 (figure 1a).

Apheresis platelet aggregated more poorly to collagen requiring 5x the concentration and are only partially inhibited by tirofiban, eptifibatide or AP-2 (figure 1b).

Lyophilized human platelets largely do not aggregate to collagen, and this is unchanged by tirofiban, eptifibatide or AP-2 (figure 1c).

Under shear force on the Total-Thrombus Analysis System (T-TAS) normal PRP occluded the channel at 10 minutes which is prevented by tirofiban, eptifibatide and AP-2 (figure 2a).

In the T-TAS system lyophilized human platelets in plasma occlude the channel at 10 minutes and are not inhibited by tirofiban, eptifibatide or AP-2 (figure 2b).

In-vitro apheresis platelets (figure 3a) were compared to lyophilized human platelets (figure 3b) for their ability to restore hemostasis in platelet rich plasma samples inhibited by tirofiban, eptifibatide and AP-2 on the T-TAS system.

In the apheresis platelet experiments the area under the curve decreased from 1049+/-316 to 393+/-294 with tirofiban; to 415+/-422 with eptifibatide, 419+/-159 with AP-2. Apheresis platelets partially restored the values to normal levels with each drug, 471+/- 303 with tirofiban; 915+/- 658 with eptifibatide and 1204+/-133 with AP-2

In the lyophilized platelet experiments area under the curve values decreased from 1290 +/- 240 to 178+/- 117 with tirofiban; 247+/- 117 with eptifibatide and 449+/-478 with AP-2. Lyophilized platelets restored the values to normal levels in the presence of each drug, 1334+/- 76 with tirofiban; 1540+/- 70 with eptifibatide and 1471+/-41 with AP-2

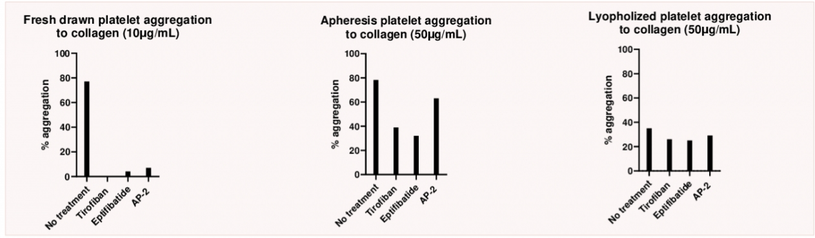

Figure 1. ***Aggregation in response to collagen.*** *(A) Fresh drawn platelets aggregate in response to 10µg/mL of collagen which is inhibited by tirofiban 100ng/mL, eptifibatide 6µM & AP-2 10µg/mL. (B) Apheresis platelet respond to 50µg/mL collagen (not 10µg/mL data not shown) which is partially inhibited by drugs. (C) Lyophilized platelets partially aggregate to collagen but no change with drugs.*

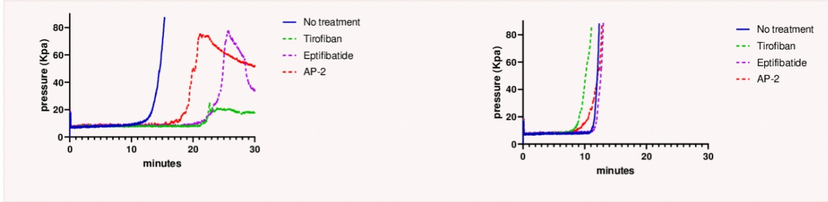

Figure 2. ***Total-thrombus analysis system*** *(A) Fresh drawn platelets occlude a tissue factor/collagen coated channel which is inhibited by tirofiban 100ng/mL, eptifibatide 6µM & AP-2 10µg/mL. (B) Lyophilized platelet occlude the channel but are not inhibited by any drug tested.*

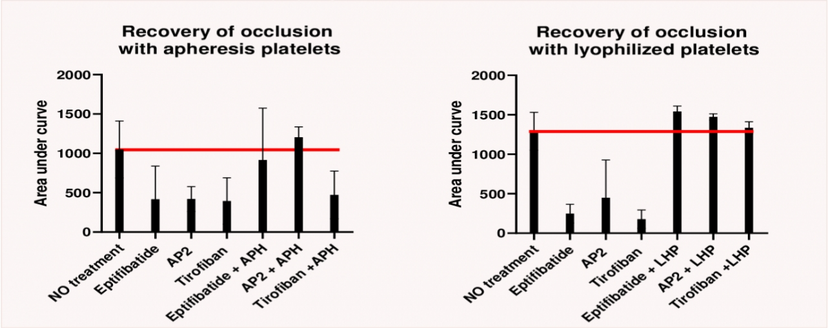

Figure 3. ***Total-thrombus analysis system, area under the curve*** *(A) Fresh drawn platelets occlude a tissue factor/collagen coated channel which is inhibited by tirofiban 100ng/mL, eptifibatide 6µM & AP-2 10µg/mL. Addition of 150k/µL apheresis platelets partially but non-reliably restored hemostasis. (B) Fresh drawn platelet again were inhibited to occlude with drugs tested but hemostasis was reliably restored with 150k/µL of lyophilized platelets.*

CONCLUSIONS

GpIIb/IIIa inhibitors are a highly selective platelet inhibitors used clinically to prevent unwanted thrombosis, but their use can result in bleeding. These bleeding events are treated by administering apheresis platelets but with marginal success. Here we have demonstrated in-vitro that lyophilized platelets are a more reliable treatment source to restore hemostasis when platelets inhibitors tirofiban, eptifibatide and AP-2 are associated with bleeding.

CONFLICTS OF INTEREST

Matthew Dickerson and Keith Moskowitz are employees of Cellphire, Inc. holding stock and/or stock options.

CONTACT INFORMATION

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