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Donor variation in the interaction of activated recombinant factor VII (rFVIIa) with human platelets and detection of a high rFVIIa binding subpopulation

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Introduction

rFVIIa is used to treat hemophilia patients with inhibitors. The need for high therapeutic doses of rFVIIa is mainly due to weak surface interaction properties requiring high concentrations for tissue factor independent FX activation on platelet surfaces. rFVIIa preferentially binds to "coated" platelets, characterized by the exposure of fibrinogen and other procoagulant proteins. Inter-individual patient characteristics in forming this platelet sub-population as well as other platelet features are known to influence bleeding phenotype.

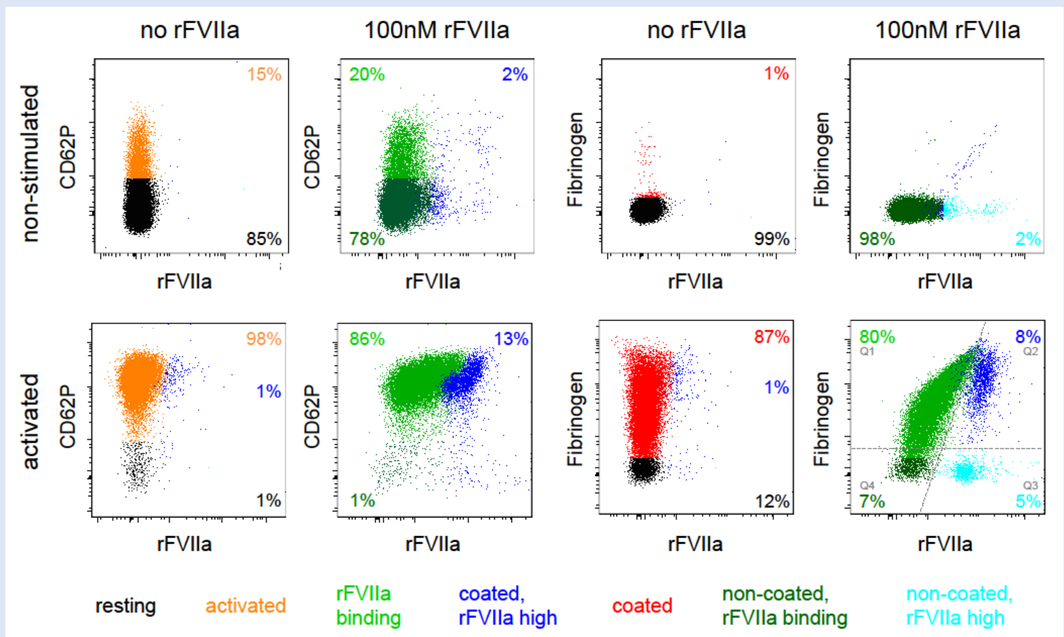
Objective

We studied the binding of rFVIIa to platelets from healthy donors by flow cytometry to characterize inter-individual differences in coated platelet formation and rFVIIa binding.

Methods

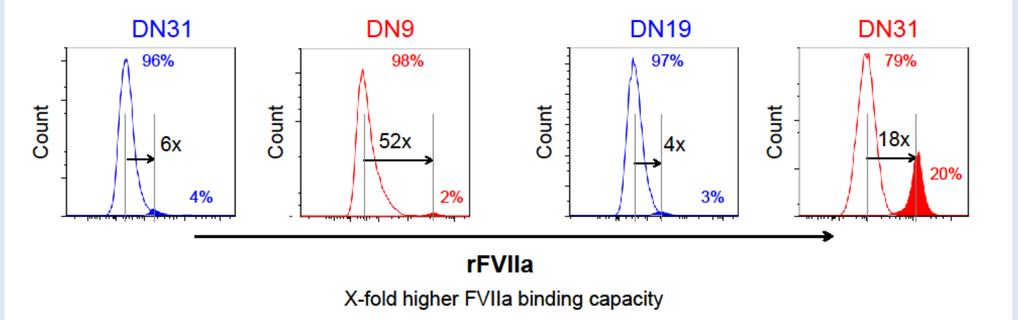
Fresh platelet concentrates from 37 healthy donors were incubated with rFVIIa at concentrations of 50 to 2000 nM for 7 min at 37°C with or without platelet activators thrombin and convulxin. Platelets were detected by staining for CD61; P-selectin and fibrinogen were used as markers for platelet activation and coated platelet formation. Bound rFVIIa was quantified using a fluorescent-labeled anti FVII antibody. Median fluorescence intensities (MFI) of platelets without addition of rFVIIa served as controls (Figure 1). For statistical comparison the MFI for the treated sample was expressed in relation to the control sample [x-fold MFI]. When a high rFVIIa binding subpopulation comprising at least 2% of the total platelet population was detected, its MFI was compared to that of the main rFVIIa binding population (Fig. 2). To estimate the activation induced rFVIIa binding capacity increase, the MFI ratio of activated and non-stimulated samples was calculated (representative examples in Fig. 4A).

Figure 1: rFVIIa staining reveals high rFVIIa binding subpopulation among coated and non-coated activated platelets



Co-staining of a typical donor for CD62P, fibrinogen and rFVIIa; the percentage of total platelets is indicated for the different subpopulations when exceeding 1%.

Figure 2: rFVIIa capacity of high binding vs major platelet population

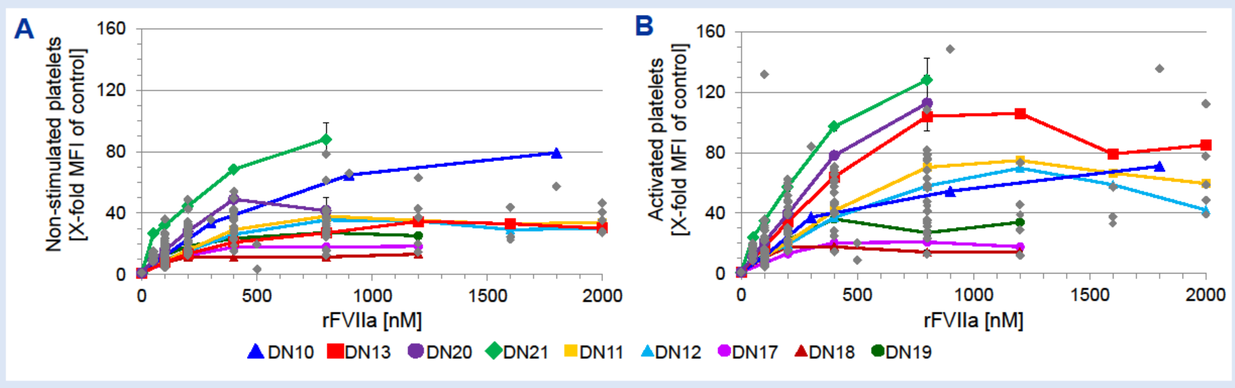


A high binding subpopulation (filled histograms) bound up to 50-fold more rFVIIa on non-stimulated and up to 18-fold more on dual-agonist activated platelets than the major population (open histograms).

Results

rFVIIa binding to non-activated and dual agonist activated platelets was concentration dependent, and mostly saturated at rFVIIa concentrations of 200 to 1200 nM. Binding constants for activated platelets ranged from 36 to 632 nM, and from 56 to 897 nM for non-stimulated platelets. Upon activation in the presence of 100 nM rFVIIa, the median fluorescence intensity increased 5- to 132-fold (mean ±SD: 15 ±16) over control cells without rFVIIa (Fig. 3B).

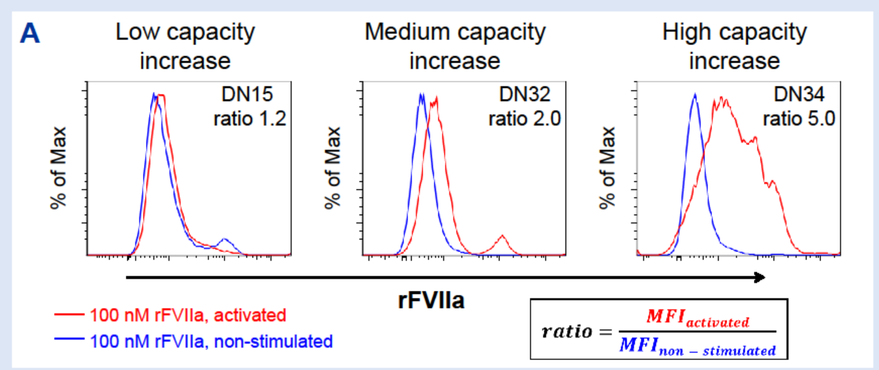
Figure 3: rFVIIa binding properties in all 37 donors (9 DN high-lighted in color)

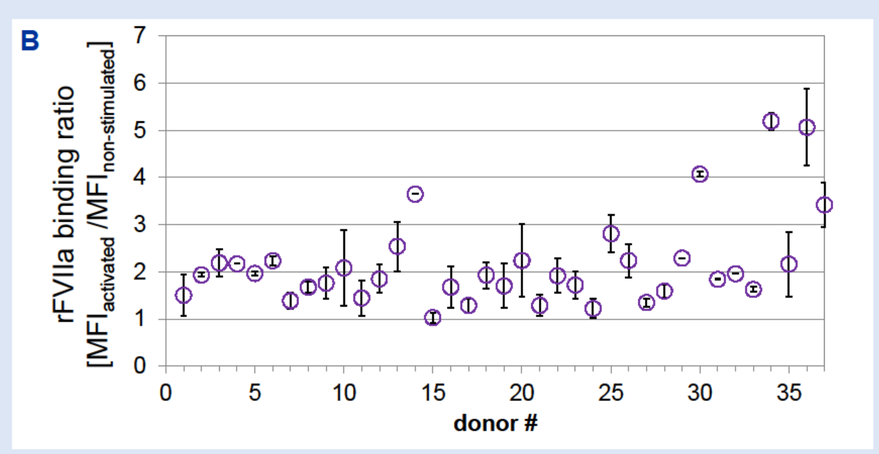


Donor-specific rFVIIa binding pattern before (A) and after (B) activation is observed.

In most donors, dual-agonist activation increased the binding capacity of rFVIIa on average more than 2-fold (Figs. 3 and 5), except in 8 of 37 donors, where the increase was less than 1.5-fold.

Figure 4: Increase in rFVIIa binding capacity upon platelet stimulation

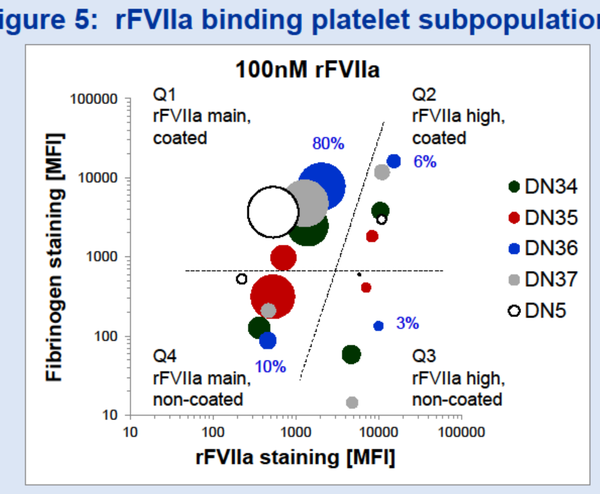




(A) Analysis of non-simulated (blue) and activated platelets (red) for rFVIIa (100nM) binding. Increase in rFVIIa binding capacity upon platelet activation is calculated and indicated. (B) For each donor the binding ratio was calculated for the individual rFVIIa concentrations and then averaged to give the donor specific response shown as mean ±SD.

A subpopulation of up to 38% of all platelets with high rFVIIa binding capacity was detected in activated and non-activated platelet samples. The formation of this subpopulation was donor specific. After dual agonist activation, platelets of this population expressed P-selectin and fibrinogen levels similar to the major platelet population (Figs. 1 and 5). In platelets activated with dual agonists and rFVIIa four distinct sub- populations can be observed in most donors.

Figure 5: rFVIIa binding platelet subpopulations



The bubble size corresponds to the percentage of all platelets, its location to the staining intensity for Fibrinogen and rFVIIa.

Conclusions

- **Considerable inter-individual variation in binding of rFVIIa to resting and activated platelets was observed**
- **We identified a platelet subpopulation of high rFVIIa binding capacity in >80% of donors.**
- **This high rFVIIa binding subpopulation consisted (up to 38 %) of "coated" and "non-coated" platelets**
- **The correlation of platelet subpopulation or binding capacity increase with treatment response should be evaluated in the future**

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If you have any additional questions, please feel free to contact Baxter Bioscience Medical Information at medinfo@baxter.com.

Conflicts of interest: The authors of this presentation make the following disclosure of financial or personal relationships with commercial entities that may have a direct or indirect interest in the subject matter of this presentation: All authors are full time employees of Baxter Innovations GmbH, Austria.

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Clotting Factor Concentrates

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