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POSTER ABSTRACTS

322.DISORDERS OF COAGULATION OR FIBRINOLYSIS: CLINICAL AND EPIDEMIOLOGICAL

Impact of Recombinant Factor VIII and Platelet Interaction on Platelet Functionality and Hemophilia a Treatment

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Background: Platelets play an important role throughout the various stages of hemostasis. After vascular injury, platelets become activated in a pro-aggregatory state, and subsequently a subpopulation undergoes a phenotype shift to a pro-coagulant state. Pro-coagulant platelets bind to factor VIII (FVIII) and ensure efficient localization of FVIII at the site of injury. It is unclear whether modifications in recombinant FVIII (rFVIII) products impact FVIII-platelet binding and subsequent platelet signaling.
Aims: To examine binding of different rFVIII products to platelets *in vitro*, and the impact of rFVIII on platelet phenotype and intracellular signaling.
Methods: *Platelet activation:* Platelets isolated from healthy donors were activated with thrombin and cross-linked collagen-related peptide (CRP-XL). *Platelet-FVIII binding*: Activated platelets were incubated with simoctocog alfa, efmoroctocog alfa, rurioctocog alfa pegol or damoctocog alfa pegol. Binding was quantified by flow cytometry either by immunostaining with an anti-FVIII antibody conjugated to Alexa fluor (AF)647 or direct measurement of rFVIII concentrates labelled with AF647. *Phenotype shift*: During the shift to a pro-coagulant state, phosphatidylserine (PS) becomes exposed on the platelet membranes, with the extent of PS exposure correlating with the level of FVIII binding. To assess PS exposure, activated platelets were incubated with Annexin V-BV421, a molecular probe with high affinity for PS. *Inhibition of integrin αIIbβ3 signaling:* Activated platelets were incubated with the integrin αIIbβ3 inhibitor antibody 10E5.
Results: Binding to pro-coagulant platelets was significantly higher with simoctocog alfa than with efmoroctocog alfa, rurioctocog alfa pegol or damoctocog alfa pegol irrespective of immunostaining method ($p<0.05$; Figure 1 shows detection with anti-FVIII antibody conjugated with AF647).To validate the variability in rFVIII-platelet interactions observed, we performed a dynamic platelet-binding assay with the two FVIII products exhibiting the strongest binding (simoctocog alfa and efmoroctocog alfa). The results of the dynamic assay confirmed the results of the static experiments (Figure 1). Exposure of activated platelets to simoctocog alfa and efmoroctocog alfa resulted in increased PS exposure, with a greater effect observed with simoctocog alfa, consistent with the results of the binding experiments. Immunostaining experiments revealed co-localization of simoctocog alfa and clusters of integrin αIIbβ3 in pro-aggregatory platelets. Inhibition of integrin αIIbβ3 by 10E5 decreased binding of simoctocog alfa to platelets and subsequent PS exposure in a dose-dependent manner (Figure 2). Analysis of platelet binding and phenotype shift in patients with hemophilia A are ongoing.
Conclusion: Simoctocog alfa demonstrated higher binding to activated platelets *in vitro* compared with efmoroctocog alfa, rurioctocog alfa pegol or damoctocog alfa pegol, resulting in an increased phenotype shift of platelets from the pro-aggregatory to the pro-coagulant state. The increased binding of simoctocog alfa was associated with a phenotypic shift in platelets as evidenced by increased exposure of PS on the platelet membranes. The binding of simoctocog alfa to platelets was disrupted when integrin αIIbβ3 activation was inhibited, suggesting a role of integrin αIIbβ3 signaling following binding of FVIII to platelets. Variations in platelet binding and signaling between different rFVIIIs might impact their efficacy for the prevention of bleeds. Further experiments are ongoing to assess the impact of FVIII on the release of von Willebrand factor and thrombin generation by activated platelets, and to repeat the FVIII-binding experiments using platelets from healthy donors vs hemophilia A patients.

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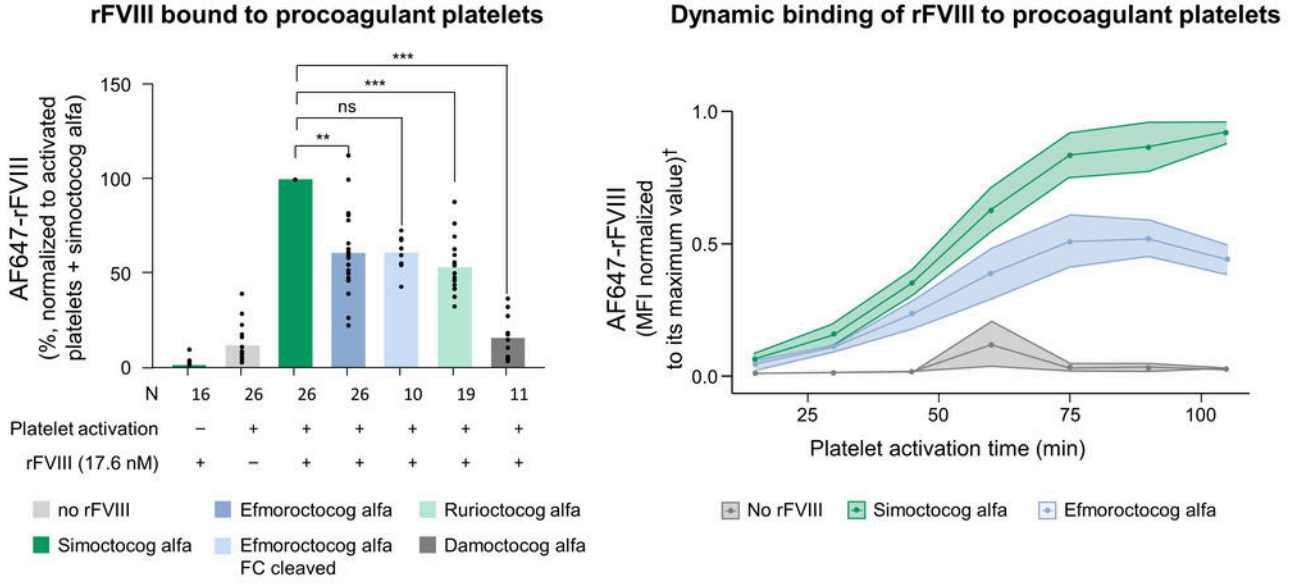
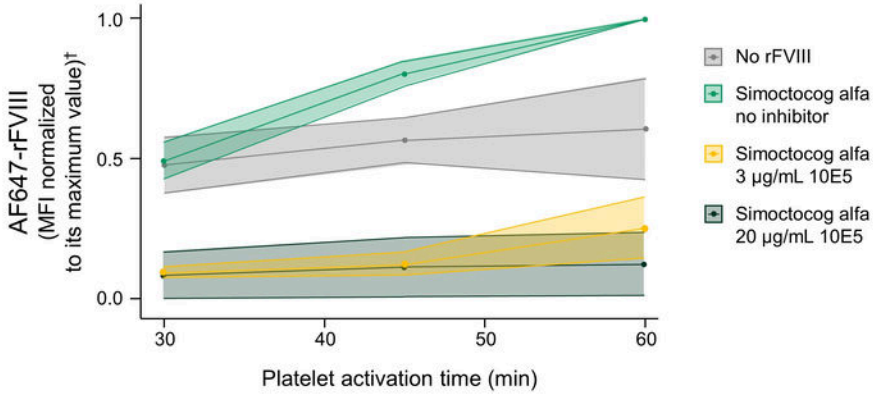
Figure 1: Simoctocog alfa shows increased binding to activated platelets vs other rFVIII factors



† Data based on samples from 6 healthy donors.
AF647-rFVIII: recombinant factor VIII immunostained with Alexa Fluor 647; MFI: mean fluorescence intensity; ns: non-significant.
Non-parametric Friedman test: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001

Figure 2: Binding of simoctocog alfa to platelets leads to increased phosphatidylserine exposure, which is blocked by inhibition of integrin αIIbβ3 signaling



† Data based on samples from 6 healthy donors.
10E5: integrin αIIbβ3 inhibitor; AF647-rFVIII: recombinant factor VIII immunostained with Alexa Fluor 647; MFI: mean fluorescence intensity.

Figure 1

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